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FORAGE QUALITY EVALUATION USING DIFFERENTIAL SCANNING CALORIMETRY AND THERMOGRAVIMETRIC ANALYSIS TECHNIQUES

by

JOHN WALSHAW

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

in

ANIMAL NUTRITION

DEPARTMENT OF AGRICULTURAL, FOOD AND NUTRITIONAL SCIENCE

EDMONTON, ALBERTA

SPRING 1996

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Degree: Master of Science

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled FORAGE QUALITY EVALUATION USING DIFFERENTIAL SCANNING CALORIMETRY AND THERMOGRAVIMETRIC ANALYSIS TECHNIQUES submitted by JOHN WALSHAW in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

Dr. G.W. Mathison

Dr. J.J. Kennelly

Dr. D.J. Pluth

Dec 19, 1995

DEDICATION

I dedicate this thesis to the memory of Peter Nesbitt and Jack Walshaw, whose selfless attitude, pragmatism and spirit of adventure have served as an inspiration to me.

ABSTRACT

Differential scanning calorimetry (DSC) and thermogravimetry (TG) were used to estimate forage chemical composition and cattle intake and digestibility parameters. Ninety three hay samples were assayed using DSC. Calibration equations developed for 56 samples were then validated using the remaining 37 samples. Predictions for crude protein and neutral detergent fibre demonstrated the greatest accuracy (R^2=0.61 and 0.72 respectively). Intake and digestibility were not well predicted by DSC (eg. R^2=0.13 for both dry matter intake and dry matter digestibility). Analysis of 18 hay samples in a preliminary study using two different TG procedures gave results that were not always consistent with each other or with the DSC study. A greater proportion of the variation in intake and digestibility measurements was explained by chemical analysis when equations developed from multiple regression analysis rather than simple regression analysis were used for prediction purposes. Thermal analysis does not appear to have potential for the evaluation of forage nutritive value.

ACKNOWLEDGEMENTS

There are many people to whom I must express my sincere appreciation. I must first of all mention my family, back in England, whose love and encouragement have meant a great deal to me during my stay abroad. I am especially grateful to my very close friends Valerie Melnychuk and Orysia Dmytruk and their families for making me feel so welcome in Canada. I would like to thank my supervisor and friend Dr. Gary Mathison for his advice and support throughout my degree programme. I also wish to acknowledge Rotary International for awarding me an Ambassadorial Scholarship and would like to thank Dr. Jerome Martin and his family for their hospitality and assistance with matters pertaining to the scholarship programme in Canada. I am very grateful to Dr Frank Robinson and his graduate students for allowing me to share their office and computer facilities and for their helpful comments and their friendship.

Mr. T. W. Fenton and Mr. G. Sedgewick must be acknowledged for their preliminary work relating to this project. I thank Mr. J. Bourgois for his invaluable assistance with the differential scanning calorimetry work. Thanks must also be expressed to A. Iacchelli and R.M. Elofson for undertaking the normal thermogravimetric measurements, and to R.L. Blaine for carrying out the high resolution thermogravimetric analysis. I would also like to thank R. Weingardt for his suggestions and assistance with the statistical analysis, and to Dr. H. Hsu and G. Recinos-Diaz at Alberta Agriculture for enabling us to analyse our data using software developed for the NIRS procedure.

Finally, I would like to thank the Alberta Agricultural Research Institute for providing the funding for this project.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
CP	Crude protein
DDM	Digestible dry matter
DEI	Digestible energy intake
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DOMD	Digestible organic matter digestibility as a % of dry matter
DOMI	Digestible organic matter intake as a % of dry matter
DSC	Differential scanning calorimetry
DTA	Differential thermal analysis
GE	Gross energy
HRTG	High Resolution™ thermogravimetry
MADF	Modified acid detergent fibre
MSR	Modified stepwise regression
NDF	Neutral detergent fibre
NIRS	Near infrared reflectance spectroscopy
NTG	Normal thermogravimetry
OM	Organic matter
OMD	Organic matter digestibility
PLSR	Partial least squares regression
r^2	Coefficient of determination (simple regression)
R^2	Coefficient of determination (multiple regression)
RMSE	Root mean square error
SD	Standard deviation
SE	Standard error
SEP	Standard error of prediction
TG	Thermogravimetric

1.0 INTRODUCTION

Domesticated ruminant livestock provide the human population with around 70% of the total amount of animal protein consumed, in terms of meat and milk consumption, in addition to their roles as a source of natural fibres and as draught animals (Minson,1990). The significance of ruminant animals to mankind, however lies in their ability to convert relatively inexpensive and plentiful fibrous plant material into commodities that can be directly or indirectly beneficial to human beings.

Forages utilised in animal production systems are tremendously variable with regard to their chemical composition and digestibility (Gill et al.,1989). These factors, along with intake potential, are the principal determinants of the nutritional and economic value of a particular forage and must be determined accurately in order to formulate rations which match animal requirements in a cost effective manner. The ability to predict forage quality, particularly in terms of its useful energy content and the amount of the feed the animal will consume voluntarily, is therefore fundamental to any forage evaluation program (Mathison,1990).

Whilst it is widely recognised that feeding trials provide the most accurate estimations of forage quality, it is obviously not feasible to use them for routinely evaluating forages due to the time required to undertake such trials and the expense involved (Marten,1981, Coelho et al.,1988). Much research attention has therefore been focussed on the development of laboratory techniques which can be used to predict the nutritive value of forages and relate these to animal performance parameters (Barnes and Marten,1979). Quality attributes of

forages, such as digestibility, are not strictly chemical in nature however, since biochemical and physical factors also come into play (Murray,1988).

Traditionally chemical analysis techniques have been used to predict forage quality. It has, however, been demonstrated that neutral detergent fibre (NDF) is not a reliable method for estimating voluntary intake (Reid et al.,1988; Minson,1990; Harlan et al.,1991). Similarly, acid detergent fibre (ADF) has been shown to be a poor predictor of forage digestibility (Reid et al.,1988; Givens et al.,1989; Barber et al.,1990). Near infrared reflectance spectroscopy (NIRS) has proven to be a useful procedure for estimating forage parameters (Norris et al.,1976; Redshaw et al.,1986; Coelho et al.,1988; Baker et al.,1994). Nevertheless, NIRS has been criticised for its inability to relate the physico-chemical properties of forage to forage quality attributes (Van Soest,1982).

Two distinct thermal analysis procedures commonly used for the analysis of materials are differential scanning calorimetry (DSC) and thermogravimetry (TG). Differential scanning calorimetry (DSC) is a technique that records the amount of energy that must either be supplied to or withdrawn from a sample in order to maintain a zero temperature differential between the sample and a thermally inert reference material whilst the sample and reference are heated according to a pre-determined temperature programme (Daniels,1973). The rate of energy absorption by the sample is proportional to its specific heat, thus any transition occurring during heating resulting in a change in specific heat is recorded as an endothermic or an exothermic enthalpy change. Thermogravimetric (TG) analysis is a technique whereby the weight of a substance is recorded as a function of

temperature or time as a sample is heated or cooled under defined conditions (Keattch and Dollimore,1975, Earnest,1988).

The DSC technique, in particular, has been used to examine the compositional changes that occur in foods during heating. Starch gelatinisation reactions and protein denaturation have received much attention (Lund,1983; Biliaderis,1983). Both TG and DSC, either individually or in combination, have for example, been used to analyse samples of wood (Reh et al.,1986; Bilbao et al.,1989b; Campanella et al.,1991), and straw (Lipska-Quinn et al.,1985; Bining et al.,1987; Bilbao et al.,1989; Sharma,1990; Ergudenler and Ghaly,1992). A number of these studies were undertaken to investigate the effects of the biological degradation of these lignocellulosic materials. Differences in the thermal curves between virgin and partially degraded materials were attributed to semi-quantitative changes in the cellulose, hemicellulose and lignin components. The quantitative analysis of 15 physical and chemical constituents found in peats has been attempted recently using thermal analysis methods and was shown to achieve the same degree of prediction accuracy as that attained using NIRS (Bergner and Albano,1993).

Forage quality evaluation using multivariate techniques, such as NIRS, have advantages over individual chemical constituent analyses because they contain information on the chemistry of the entire feed and not isolated constituents within it (AFRC.,1991). The application of thermal analysis techniques to forage quality evaluation, using the same approach as that detailed in the pioneering NIRS study by Norris et al. (1976), may provide a more direct estimate of forage nutritive value than is currently possible with NIRS.

2.0 LITERATURE REVIEW

2.1 Forage Quality

Forage quality and feeding value are terms used synonymously to describe the potential of a forage to meet the nutritional requirements of livestock and are defined by Barnes and Marten (1979) as: "the type and amount of digestible nutrients available to the animal per unit time". Chemical and physical characteristics of forages are affected by a multiplicity of factors which include forage species and variety, and environmental and management factors such as nutrient status of the sward and the growth stage and growth rate of the crop at the time it is either cut or grazed (Gill et al.,1989; Beever,1993).

The plant cell wall component consists mainly of hemicellulose, cellulose and lignin (Marten,1981; Beever,1993). Quantities of hemicellulose, cellulose and lignin all increase as forages mature, although legumes consistently show lower proportions of hemicellulose and higher proportions of lignin than grasses harvested at a similar growth stage (Barnes,1973; Osbourne,1978; Gill et al.,1989). Lignin is of particular significance since this component is indigestible and serves to reduce the digestibility of cellulose and hemicellulose (Van Soest,1965; Beever,1993) which could otherwise be completely digested by ruminants (Minson,1982).

2.2 Prediction of forage quality from chemical analysis

Historically, the Weende proximate analysis system formed the basis for estimating forage chemical composition, with the crude fibre fraction purportedly representing the indigestible portion of the feed (Van Soest,1968). Crude fibre analysis was widely criticised because of inherent theoretical methodological inaccuracies (Crampton and Maynard,1938; Van Soest,1968; Barnes,1973). In a review of experimental results Minson (1982) demonstrated r^2 values ranging from 0.09 to 0.70 and from 0.41 to 0.88 when predicting forage dry matter digestibility (DMD) and organic matter digestibility (OMD) respectively from crude fibre measurements.

Detergent fibre analysis (Goering and Van Soest,1970) has now superseded crude fibre as a means of predicting forage digestibility (Marten,1981). In the UK, a modified acid detergent fibre analysis (MADF) (Clancey and Wilson,1966) which uses a longer hydrolysis period has been routinely used to predict forage DMD. This approach has however been criticised for undermining the chemical basis of the ADF determination (Van Soest and Mertens 1977). In a survey of American researchers, acid detergent fibre (ADF) and neutral detergent fibre (NDF) were deemed to be the preferred techniques for estimating DMD and dry matter intake (DMI) respectively (Rohweder and Baylor,1980). Minson (1982) considered that the OMD of grasses and legumes can be predicted with a similar accuracy using NDF and ADF but recommended the use of ADF because, unlike NDF, no apparent large differences occur between regressions for grass and legumes. The relationship between digestibility and ADF is confounded by a number of factors including forage species and

environment (Osbourne,1978; Van Soest,1982). For this reason, Van Soest (1982) emphasised that relationships between ADF and digestibility are merely statistical in nature. Van Soest et al. (1978) postulated that ADF could not be used to accurately determine the digestibility of aftermath forages because in such samples no positive relationship between cellulose and lignin was apparent. Also, the range in ADF values was small in comparison with those observed from primary growth forages which resulted in a poorer correlation with digestibility. Similarly, Givens et al. (1993) were unable to satisfactorily predict the in-vivo digestible organic matter contents (DOMD) of autumn regrowths from acid detergent measurements.

Updated prediction equations for DOMD, routinely used by the extension service in the UK were reported by Barber et al.(1984). Estimates of DOMD from MADF and ADF analysis respectively had R^2 values of 0.56 and 0.39 for fresh grass, 0.73 and 0.70 for hay, 0.54 and 0.48 for silage, 0.46 and 0.11 for dried grass and 0.70 and 0.75 for dried alfalfa. Minson (1982), in a comprehensive review of the literature demonstrated r^2 values ranging from 0.23 to 0.92 for the prediction of DMD and 0.31 to 0.86 for the prediction of OMD, from ADF. Results from similar recent studies have been equally variable and often poor. Reid et al.(1988) in a retrospective study covering a 20 year period and using data on 428 forages fed to sheep and 170 forages fed to cattle, found that the regression equations relating ADF to DMD for cattle and sheep respectively explained 3% and 38% of the variation for temperate grass, 0% and 15% of the variation for legumes and 23% and 45% of the variation for tropical grasses. Mathison (1990) observed that ADF could only account for between

34% and 58% of the differences in DE content of grass and legume hays.

Other researchers have estimated in-vivo OMD from ADF and found that ADF explained 53% (Aerts et al.,1977) and 44% (Aufrere and Michalet-Doreau,1988) of the variability in the OMD content of forages and a broad range of feeds including forages in their respective studies. In two recent experiments MADF has been regressed against OMD using in excess of 120 silages and only 34% (Barber et al.,1990) and 56% (Givens et al.,1989) of the variation in OMD could be accounted for by the MADF data. Coelho et al. (1988), however were able to explain 71% of the variation in DMD of hay samples from the ADF content, but only six hays were used in this experiment. Correspondingly, Miller et al. (1991) reported R^2 values ranging from 0.57 to 0.79 relating the ADF content of cubed or pelleted alfalfa diets to cattle and sheep.

Subdivision of the error terms associated with the prediction of digestible dry matter (DDM) from ADF analysis showed that the largest proportion, 68%, was due to the lack of fit of the equation, meaning that forage ADF could not adequately account for the observed differences in DDM (Abrams,1988). This study also demonstrated that between animal variation, within laboratory and between laboratory variation accounted for 6%, 0.5% and 5% of the remaining variation, thus indicating that improving the accuracy of ADF determinations is unlikely to lead to significant improvements in DDM prediction.

It is clear from the literature that ADF analysis, despite its widespread adoption, provides a less than satisfactory estimate of forage digestibility. Consequently, it has been recommended that its value is limited for the prediction of the digestibility of hays

(Mathison,1990), fresh herbage (Givens et al.,1990), and silages (Barber et al.,1990). In addition, Weiss (1993) also considered that the estimation of the available energy content of forages based on ADF lacked precision.

Voluntary intake of ruminant animals fed forage based diets has often been suggested to be best estimated from NDF analysis (Van Soest,1965; Van Soest and Mertens,1977). The theoretical premise for using NDF to predict voluntary intake is that NDF is correlated with forage bulk density, which in turn has a negative effect on intake. The NDF content of particles that are slowest to leave the rumen is also higher, and these particles require a longer rumination time for particle size reduction (Meissner et al.,1989).

Van Soest (1965) considered that NDF could explain 76% of the variation in voluntary DMI. Unfortunately, other workers have reported a variable and often poor relationship between these parameters. Hoover (1986) in a review of data from lactating dairy cattle suggested that only 58% of the variability in DMI could be accounted for by NDF values of all forage diets and this figure fell to 33% for grain plus forage rations.

A comprehensive review of the literature by Minson (1990) demonstrated r^2 values ranging from 0.14 to 0.81 using data compiled from a wide range of forages. In a retrospective 20 year study of forages fed to sheep and cattle it was considered that forage NDF content could only explain 5% and 29%, 35% and 18%, and 22% and 26% of the differences in observed DMI for temperate grass, legume and tropical grass forages, respectively (Reid et al.,1988). Mathison (1990) in a study using 114 forages reported that NDF only accounted for 8%, 24%, and 6% of the variation in DMI ($g\ kg^{0.75}$) for alfalfa,

legume-grass and grass samples respectively. Other reports have shown r^2 values of 0.09 to 0.46 between NDF and DMI /100kg body weight in non-lactating dairy cattle (Harlan et al.,1991) and r^2 values of 0.27 to 0.76 from regression equations relating herbage NDF content to organic matter intake in sheep (Meissner et al.,1989).

The experiment of Harlan et al. (1991) actually found ADF to be more closely related to ad-libitum DMI than forage NDF content. These authors went on to speculate that the use of NDF to predict DMI for dairy cattle had merely been recommended due to the relationship between diet NDF and the ration forage:concentrate ratio. These workers also postulated that the cellulose content of forages may be the most beneficial fibre component for the estimation of the voluntary intake of hays.

Even though the NRC (1988) contends that NDF is related to DMI, it is obvious that an improved method for predicting the intake potential of forages is highly desirable.

2.3 Prediction of forage quality from Near Infrared Reflectance Spectroscopy (NIRS)

Near infrared reflectance spectrscopy (NIRS) has become an accepted technology for the rapid, cheap reliable and non-consumptive evaluation of organic material (de Boever et al.,1995). The theoretical basis for NIRS is that energy reflected from the sample is related to its physical and chemical makeup (Osbourne and Fearne,1986; Baker and Barnes,1990). The shape of the NIR spectra contains information regarding the amount of hydrogen that is bonded to carbon, oxygen or nitrogen. In practice, the reflectance spectrum of a feed is scanned using a spectrophotometer. A microcomputer is used to correlate this scan with

those scans of known composition that have previously been used to calibrate the machine, and hence to predict the composition of the unknown sample.

Norris et al. (1976) were the first to use NIRS technology for the purpose of predicting forage quality parameters. These workers reported some very encouraging results, with estimated R^2 values of 0.96 (SE = 1.07) for crude protein, 0.85 (SE = 5.3) for NDF, 0.81 (SE = 2.5) for ADF, 0.52 (SE = 2.1) for lignin, 0.65 (SE = 4.4) for DMD and 0.62 (SE = 7.8) for intake using 87 samples representing a wide range of forage types.

In a 1983 study, Minson et al. using a fixed filter NIRS instrument to predict DMD and voluntary intake in 97 tropical forages obtained standard errors of prediction of 0.27% and 7.2 kg $kgBW^{0.75}$ respectively, however these standard errors were substantially reduced when the large biases due to grass species, plant part and physical form of the feed were taken into account. Winch and Major (1981) also working with fixed filter NIRS equipment were unable to estimate in-vivo digestibility of grasses and legumes to an adequate degree of precision. Consequently the contention has been made that scanning NIRS instruments are superior to fixed filter ones due to their increased flexibility (Marten, 1989).

Other studies using scanning NIRS technology have reported results that are in agreement with the original work of Norris and co-workers. Burdick et al. (1981) considered that the standard errors of 1.78% to 2.54% they achieved for estimating the DMD of forages fed to sheep from NIRS were somewhat lower than those calculated from more conventional laboratory procedures. Reeves (1988) considered that various chemical methods of fibre analysis (NDF, ADF and lignin) could be accurately assayed to a high degree of precision

with NIRS. Coelho et al. (1988) similarly demonstrated a strong association between NIRS data and laboratory measurements conducted on hay samples (R^2 = 0.97, 0.98, 0.96 and 0.73 for CP, NDF, ADF and lignin respectively). Dry matter intake, digestible energy and DMD were also found to be closely related to NIRS measurements in this study, with R^2 values of 0.84, 0.75 and 0.69 respectively being reported. These workers also compared the NIRS results with those from chemical analysis and found NIRS superior to NDF and ADF though not to CP with regard to the prediction of intake. Near infrared reflectance spectroscopy measurements were more poorly related to DMD than were CP and ADF values.

Analysing a range of 15 forages using NIRS, Lippke and Barton (1988) suggested that there was a strong correlation between NIRS measurements and protein, NDF and ADF content of the feed, as well as between the DDM, digestible organic matter intake (DOMI), and average daily gain of cattle. These authors went on to postulate that the prediction of DOMI from NIRS data may be justifiable from a biological standpoint.

A comparative study of techniques aimed at predicting silage OMD found that NIRS using eight wavelengths in the prediction equation developed on a calibration set could explain 76% of the variability in OMD in an independent validation set (Barber et al.,1990). Comparable R^2 values for other techniques evaluated in this experiment were 0.20 for MADF, 0.40 for enzyme degradability and 0.64 for in-vitro digestibility measurements, leading the authors to the conclusion that NIRS is sufficiently accurate for routine use.

Alberta forages have also been analysed by NIRS to estimate their chemical composition, intake potential and digestibility in both cattle and sheep (Redshaw et al.,1986). Results

estimating chemical composition parameters from NIRS in this study were generally in agreement with those of other workers in terms of both primary wavelength selection and prediction accuracy (R^2 = 0.95, 0.71 and 0.90 for the prediction of CP, ADF and NDF respectively). These researchers were also able to estimate DMD and voluntary intake (DMI $kg^{0.75}$) reasonably accurately, although animal species differences were evident (R^2 = 0.68 and 0.72 respectively for cattle and 0.47 and 0.83 respectively for sheep). Redshaw et al. (1986) emphasised the fact that when data from animal feeding trials are used in NIRS calibration procedures, between animal variation is included in the variability of prediction. They calculated that this variability was responsible for around 50% and 17% respectively, of the variation in NIRS prediction of DMI and DDM. In a subsequent study with additional Alberta forages (G.W. Mathison unpublished) found that although associations between NIRS data and chemical analysis were worse than those obtained previously, marked improvements were seen in the prediction of DMD and OMD. Voluntary intake was estimated to a similar degree of accuracy to that which had been previously achieved by other workers.

NIRS has also been used successfully to estimate forage quality parameters in small grain forages (Marten et al.,1983), subtropical forages (Synman and Joubert,1993) and tropical grasses (Purnomoadi et al.,1992). Fairbrother and Brink (1990) were able to analyse forage cell wall polysaccharides for pectin, arabinose, xylose and glucose, reporting r^2 values of between 0.84 and 0.90 for a validation data set in their study. The NIRS technique has also been recommended for the routine analysis of compound feeds for cattle (de Boever et

al.,1995).

Despite the fact that NIRS is undeniably the method of choice for the evaluation of large numbers of forage samples, it cannot be directly related to digestibility, rather calibration samples are required. This empirical approach is thus not well suited to the needs of basic research on forage composition (Van Soest 1982) or to the prediction of nutritive value across a broad range of forage species and environments. Although it has been speculated that in the future it may be possible to analyse feeds directly using only the spectral information, rather than having to calibrate technologically advanced equipment with inadequate wet chemistry data (Murray, 1988), currently NIRS must rely upon the calibration/validation approach, with data obtained by more traditional means. This approach demands that several key criteria are met. Calibration samples must be representative of the entire population whose composition is to be predicted and at least 50 samples should be used for instrument calibration (Shenk et al.,1979). In addition, predictive equations developed on calibration samples must be carefully validated using an independent set of similar forage samples (Westerhaus,1989; Barber et al.,1990).

Recently, the multivariate statistical regression procedures of modified stepwise regression (MSR) and modified partial least squares regression (PLSR) have been compared for their effectiveness in NIRS equation development (Shenk and Westerhaus,1991; Baker et al.,1994). Shenk and Westerhaus (1991) found that the validation statistics estimating CP, ADF and in-vitro dry matter disappearance gave a standard error of prediction that was 18% lower with PLSR than for MSR. Similarly, Baker et al. (1994) found that the best PLSR

regression equation used to validate NIRS predictions of silages with in-vivo OMD was six percent more accurate than the best MSR equation (R^2 = 0.82 versus 0.77 for PLSR and MSR respectively).

The NIRS technique has become accepted and recommended by the scientific community as a rapid, repeatable method for use in routine forage analysis programs. This should not, however, preclude research into other techniques which can characterise forages, since such techniques may be able to shed more light on the complex subject of the physico-chemical makeup of forages and its impact on animal intakes and resultant digestibility within the animal.

2.4 Thermal analysis

Thermal analysis is the collective term used to describe various individual techniques whereby a particular physical property of a sample and its temperature are measured continuously according to a controlled temperature program (Daniels,1973; Wendlandt,1986). Two separate methods of thermal analysis are evaluated in this thesis with respect to their ability to estimate forage quality.

2.4.1 Thermogravimetry

Thermogravimetric (TG) analysis is a technique whereby the weight of a substance is recorded as a function of temperature or time as a sample is heated or cooled under defined conditions (Keattch and Dollimore,1975; Earnest,1988). Organic materials, such as forages,

lose weight as they decompose in response to increasing temperature. Information on weight loss with heating can provide information pertaining to the composition of the sample, both initially and during decomposition, and of the remaining ash content as well as describing the thermal stability of the sample (Wendlandt,1986).

Thermogravimetric instrumentation typically includes a sensitive microbalance, a furnace incorporating an appropriate vessel in which to heat the sample, a temperature programer, a temperature measuring device, a pneumatic system to dynamically purge the sample chamber and a data acquisition system (Earnest,1988). A recent innovation has been the development of high resolution™(TA Instruments Inc.) TG (HRTG) analysis whereby high heating rates are employed in regions showing no changes in decomposition with the heating rate being adjusted smoothly and continuously in response to any changes in sample weight. Such an approach can improve the resolution of most temperature separable transitions and reduce analysis time considerably.

Improvements in TG analysis equipment have facilitated the use of this technique to solve a diverse range of analytical problems (Charsley and Warrington,1988). Compositional analysis of coal and related products, in terms of water, volatiles, fixed carbon and ash contents is probably the best known application of the TG technique for evaluating heterogenous materials (Charsley and Warrington,1988) as it can be relatively accurately and quickly performed (Cumming,1981; Wiedemann et al.,1988). The procedure has been used for the analysis of fuels, clays, inorganic compounds, polymers and pharmaceutical products (Wendlandt,1986) and in studies looking at the structure and

chemical composition of feedstuffs such as cereal, meat and dairy products (Ma and Harwalkar, 1991).

A range of factors will affect the shape of the resultant thermogravimetric curve. These include sample mass, particle size, container design, temperature measurement device location, heating rate and the atmosphere chosen (Keattch and Dollimore,1975; Wendlandt,1986). Within an experiment these variables can be readily defined, however care should be exercised when comparisons are to be made using data obtained from different studies using different instruments and experimental protocols, since all of these factors can influence the shape of the curves (Daniels,1973). Nevertheless, under specified conditions weight loss data generated from TG analysis is reproducible (Wiedemann et al.,1988; Orfao and Figueiredo,1993). Mass transfer phenomena have been said to influence the kinetics of the thermal breakdown of large particles of lignocellulosic materials, thus particle sizes of less than 1 mm are preferable (Koufapanos et al.,1989). In order to minimise the magnitude of temperature gradients occurring within the sample, small sample sizes are desirable (Keattch and Dollimore,1975) with those of approximately 10 mg generally giving optimum resolution (Daniels,1975). Different purge gas flow rates (nitrogen) did not significantly affect TG curves in studies of Balci et al. (1993).

Bilbao et al. (1989a) stated that the physical and chemical nature of lignocellulosic materials markedly affects the rate at which such materials lose weight during thermal decomposition. Thermogravimetric curves of biomass, such as wood products, peats and straws have in common two distinct reaction zones, the first rapid reaction often being

referred to as the active pyrolysis zone and a second one where much lower rates of weight loss are encountered. Different constituents of biomass, particularly cellulose, hemicellulose and lignin, exhibit different thermal properties (Shafizadeh,1985). The main components found in biomass generally behave independently and each type of chemical bond normally breaks at a different temperature (Balci et al.,1993).

Cellulose is considered to commence thermal degradation at around 300°C and progress rapidly at temperatures above this when moderate heating regimes are employed (Shafizadeh,1985; Milne,1981), with the peak transition occurring in the 300-350°C range (Julien et al.,1991). Hemicellulose is considered to decompose in a similar manner to cellulose but at lower temperatures (Ramiah,1970; Milne,1981). Lignin can also however, commence degradation at lower temperatures than cellulose or hemicellulose and does not decompose as completely as cellulose or hemicellulose. (Shafizadeh,1968; Koufopanos, 1993). Bilbao et al. (1989b), using their own data as well as that in the literature, considered hemicellulose to be largely decomposed by the time the temperature reaches 260°C, cellulose to commence decomposition at around 230°C and lignin at around 290°C. This high initial degradation temperature for lignin appears to contradict the findings of Shafizadeh (1968) however, Bilbao and co-workers attributed this phenomenon to the fact that some lignins do not decompose at low temperatures. Lipska-Quinn et al. (1985) detailed results from TG experiments carried out on holocellulose and cellulose extracted from rice straw, as well as the intact rice straw itself, using different heating rates and pyrolysis environments. In the active pyrolysis region, the cellulose degraded faster than the holocellulose, which in turn

degraded faster than the untreated rice straw.

During thermal degradation, wood samples have been said to act as if they were a mixture of cellulose, hemicellulose and lignin (Eikner,1962; Stamm,1962). Thermal curves describing biomass pyrolysis have been shown to represent the collective contribution of each of these components in the material (Shafizadeh and McGinnis,1971) . Furthermore, each component's contribution to the thermal curve is proportional to its presence in the lignocellulosic material of concern (Koufapanos et al.,1989). Nonwood plants, such as straws, generally contain greater proportions of cellulose and hemicellulose relative to lignin than wood, and their chemical composition is more closely related to that of hardwoods rather than softwoods (Nassar,1985).

Various cereal straws have been subjected to TG analysis and it seems likely that TG curves produced from other forage samples will have some similarities with these. Ergudenler and Ghaly (1992) conducted a TG study using four varieties of wheat straw heated in an oxidising atmosphere comprising 85% nitrogen and 15% oxygen. Initial decomposition temperatures for the straw varieties ranged from 205 to 222°C when a heating rate of 20°C min^{-1} was used. Differences were attributed to differences in the proportions of the main lignocellulosic components present in each straw variety. Virtually no weight losses were reported once sample temperatures reached 550°C, and around 55% of the decomposition occurred in the active pyrolysis zone and 35% in the second reaction zone.

One variety each of wheat, barley, oat and rye straws were assessed using the TG procedure with three different heating rates (10, 20 and 50°C min^{-1}) in both air and nitrogen

atmospheres in studies by Bining et al. (1987) and Ghaly and Ergudenler (1991). During the most active phase of pyrolysis, in both studies, the thermal degradation rate was generally seen to be highest in the presence of nitrogen, however the opposite was true in the passive phase. These authors also noted that initial degradation temperatures for all the straw samples at every heating rate were higher in a nitrogen atmosphere. They also speculated that the initial degradation temperature may be related to the cellulose content of the straws.

Increasing the heating rate, irrespective of the prevailing atmosphere, resulted in a greater pyrolysis degradation rate and a decreased initial degradation temperature when straw samples were analysed using TG (Lipska-Quinn et al.,1985; Bining et al.,1987; Ghaly and Ergudenler,1991). Straws having a high cellulosic content, such as those from rye, have been shown to exhibit a more rapid thermal degradation rate relative to oat, barley and especially wheat straws (Ghaly and Al-Taweel,1990; Ghaly and Ergudenler,1991).

Residual weights of straw samples after pyrolysis are greater in a nitrogen atmosphere than in an air atmosphere (Lipska-Quinn et al.,1985; Bining et al.,1987; Ghaly and Ergudenler,1991), and are also greater at higher temperatures due to the reduction in reaction time (Bining et al.,1987; Ghaly and Ergudenler,1991). Also, ash contents of straw samples have, as expected, been correlated with residual weights obtained following thermal degradation (Bining et al.,1987; Ghaly and Ergudenler,1991).

Fresh, ancient and waterlogged woods have also been analysed using TG in an air atmosphere (Campanella et al.,1991). It was contended that TG provided accurate quantitative values for the moisture, cellulose, lignin and ash contents of each class of

sample, even though the aged specimens had undergone considerable molecular degradation. Also, Sharma (1990) suggested that a combination of TG and fibre analysis may be a useful means of assessing the rate of breakdown of polysaccharides in composted materials. In addition, it has been reported that the carbohydrate content of peat samples is directly related to the TG weight losses associated with the first exothermic reaction (Almendros et al.,1982).

Most TG studies of biomass have included attempts to model reaction kinetics, the aim being to develop feasible technologies for the recovery of energy from what are essentially waste materials (Ergudenler and Ghaly,1992). Kinetic studies of weight losses occurring during thermal decomposition in a nitrogen atmosphere and a series of different heating rates (1.5°C min^{-1} to 80°C min^{-1}) were undertaken using samples of cellulose (Bilbao et al 1987a; 1987b) hemicellulose and lignin (Bilbao et al 1989a). The same workers then used this collection of kinetic data to successfully predict the kinetics of the pyrolysis of pinewood and barley straw from the weighted contributions of cellulose, hemicellulose and lignin, for both of these lignocellulosic materials (Bilbao et al.,1989b). Pyrolysis curves for the wood and straw were considerably different from one another, and the authors hypothesised that this was due to differences between the hemicellulose, fixed carbon and ash contents of the materials themselves, in conjunction with differences in the types of lignin found in wood and cereal straws. Estimations for the kinetics of decomposition were compared with the actual weight loss data for each material, and the barley straw estimated curve did not fit the data as closely as was the case with the pinewood. A conceivable explanation for this is that the individual lignocellulosic components used for the analysis and prediction were obtained

solely from wood sources (Bilbao et al.,1989).

Koufopanos et al. (1989) attempted to correlate pyrolysis rate with biomass composition and concluded that materials such as wood and corn cobs produced considerably different thermal curves than olive husks and hazelnut shells because the predominant component in each was cellulose and lignin respectively. They also attributed a portion of the weight losses to hemicellulose decomposition. Working with almond and hazelnut shells, Balci et al. (1993) demonstrated that the pyrolysis of these materials was 90% complete at 450°C when a heating rate of 20°C min^{-1} was employed. These workers also examined the kinetics of beechwood thermal decomposition and found the initial rate of carbonisation was greater for beechwood than for the shells of either almonds or hazelnuts, due to the larger proportion of holocellulose present with respect to lignin. The authors used their findings to support the hypothesis that biomass decomposition results from the break up of the weakest bonds initially, followed by the breakage of stronger bonds.

Relationships reported between TG curves of lignocellulosic materials and their respective main components strongly suggest that TG analysis may provide additional information relevant to forage quality assessment, particularly with regard to sample carbohydrate and lignin contents which influence forage intake and digestibility.

2.4.2 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) instruments record the amount of energy that must either be supplied to or withdrawn from a sample in order to maintain a zero temperature differential between the sample and a thermally inert reference material, whilst the sample and reference are heated according to a pre-determined temperature programme (Daniels,1973). The sample and reference (often an empty sample pan) are heated in identical environments with individual heaters.

Very small sample sizes are used for DSC work and a good contact surface between the sample pan and the sample itself must be achieved for optimum resolution. Samples therefore commonly take the form of fine granules, thin films or mixtures in water (Lund,1984). Obviously, when evaluating heterogenous materials small sample size can present some problems in ensuring a truly representative sample. On the other hand, DSC does facilitate a single direct assessment of complex samples, such as wood, and is less likely to introduce artefacts into the analysis than is the case with chemical methods (Reh et al.,1986).

The rate of energy absorption by the sample is proportional to its specific heat, thus any transition occurring during heating resulting in a change in specific heat is recorded as an enthalpy change (Daniels,1973). Enthalpy values for the thermal reaction of interest are obtained by measuring the area under the thermal curve. Area under the DSC curve is deemed to be less sensitive to experimental parameters such as sample size, homogeneity and heating rate, than is peak height (Reh et al.,1986) however, these operational factors can be

controlled within an experiment. Endothermic reactions are identified as upward peaks on the DSC curves and exothermic reactions as downward peaks. Instrument calibration using high purity metals with known enthalpy of fusion and melting points are used to accurately find the calibration constant and set the temperature scale (Lund,1984).

Differential thermal analysis (DTA), the parent technique of DSC, produces thermal curves that look very similar to those from DSC instruments. In DTA, actual temperature differences between the sample and reference are measured as a function of temperature. A disadvantage of this technique over DSC is that the calibration constant is temperature dependent and, although DTA provides useful information on transition temperatures, such temperatures cannot be readily converted into enthalpy change values (Lund,1984). With DSC, the differential heat inputs can be measured directly and provide more quantitative data relating to transition temperatures and complete or partial reaction enthalpies than does DTA (Lund,1983).

Both DSC and DTA have found applications in similar research areas. Biological materials such as grains, coffees, peats, biomass, protein, starch and fats and oils have all been studied by DTA and/or DSC (Wendlandt,1986). Food scientists have used DSC extensively to study the physical transitions that take place in foods during heating, providing useful qualitative and semi-quantitative information concerning these processes (Lund,1983). The processes of starch gelatinisation, protein denaturation, lipid oxidative stability and water binding have received the greatest attention (Biliaderis,1983; Lund,1983).

Starch gelatinisation is important because of its effects on the texture of starch containing

foods. The phenomenon of starch gelatinisation involves the irreversible swelling of starch granules in a aqueous medium at a specific temperature, leading to the formation of a gel-like mass (Lund,1984; Zanoni et al.,1995). The DSC technique was first employed for this purpose by Stevens and Elton (1971), although Morita (1956) used DTA curves to characterise potato and corn starch and related polysaccharrides. Examinations of the transitions occurring in starch/water systems have been carried out over a wide range of temperatures and moisture contents. Different moisture levels in these starch/water systems produce different DSC curves. Normally at high moisture levels a single endothermic peak appears at around 60°C, at intermediate moisture levels a second temperature dependent endothermic peak is observed and at low moisture levels only this second peak is apparent (Biliaderis,1983; Zanoni et al.,1995). Below 30% moisture levels gelatinisation does not take place (Collison and Chilton,1974). Attempts to characterise the relationship between the water concentration and the melting point of potato and legume starches respectively using DSC data have been made, with the starch granule being considered a typical semicrystalline polymer (Donovan,1979; Biliaderis et al.,1980). This approach was later deemed inappropriate and an alternative three phase model was proposed by Biliaderis et al. (1986). More recently, Slade and Levine (1991) postulated that starch consists of amylose and amylopectin crystals scattered throughout a non-crystalline matrix and that the gelatinisation process itself involves a transition in the non-crystalline component and melting of the crystalline component, with both components being detectable from a DSC trace. Zanoni et al. (1995) have subsequently developed a kinetic model of starch gelatinisation to be applied

to bread making based on these principles.

Effects of the hot air drying of wheat on its starch gelatinisation characteristics in terms of an increased gelatinisation temperature and an altered enthalpy of the first transition have been measured using DSC analysis (Zamponi et al.,1990). Starches isolated from different maize varieties were seen to demonstrate variation in their thermal properties when assessed using DSC and it was speculated that maize genotypes could be identified from calculated enthalpy values (Krueger et al.,1987). White et al. (1990), looking at inter- and intrapopulational differences in the thermal properties of maize starch, considered variations in DSC data to represent differences in composition and the degree of crystallinity which could have a genetic origin.

Phase transitions occurring during DSC analysis of young and mature plant cell wall preparations from soybean hypocotyls, with and without the addition of calcium were examined by Lin et al. (1991). Additions of calcium significantly altered the thermal transitions of the young but not the mature cell wall preparations and it was thought that calcium binding to pectin and strengthening the cell wall may account for this observed change. The authors also outlined other potential uses for DSC in elucidating the architecture of plant cell walls, such as examining the thermal behaviour of the cell wall with individual components (protein, hemicellulose pectin) systematically removed and then reassembled.

In the case of protein denaturation, DSC traces can be used to detect structural changes occurring within protein molecules such as melting or unfolding caused by heating, but this is not possible for proteins lacking folded secondary structures. (Ma and Harwalkar,1991).

Enthalpy of denaturation values calculated from DSC curves have been correlated with the ordered secondary structure content of a protein (Koshiyama et al.,1981). Ma and Harwalkar (1991), reviewing the role of DSC in protein analysis, demonstrated its usefulness for compositional analysis and in determining the kinetics of denaturation of muscle, egg white, and milk and vegetable proteins.

Workers have used DSC to investigate crystallisation reactions involving cellulose. Hatakeyama et al. (1976) examined crystallinity in, and the reaction kinetics of D-glucose and cellulose oligosaccharrides such as cellobiose. Kinetic analysis of cellulose and subsequent D glucose decomposition in sulphuric acid using DSC suggested that DSC could be used to estimate the yield of glucose from acid hydrolysis of the parent cellulose (Kunihisa and Ogawa,1985). A later study by these authors demonstrated that the reaction rate and enthalpy were influenced not only by the degree of crystallinity, but also the initial moisture content of the cellulose material, with the most rapid reaction rate occurring in the 13% moisture samples (Kunihisa ans Ogawa,1988). No other related studies were found in the literature.

Many studies have examined the pyrolysis of individual lignocellulosic components by DTA (Milne,1981). Shafizadeh and McGinnis (1971) found cellulose and hemicellulose decomposition to be endothermic whilst lignin decomposition was exothermic, in a comparison of cellulose, hemicellulose and lignin degradation in a nitrogen atmosphere,. Some differences in the direction of heat flows during the thermal decomposition of lignocellulosic materials have been observed. Cellulose decomposition was considered to

have both endothermic and exothermic components in a nitrogen atmosphere but in air only exothermic heat flows occurred (Ramiah,1970). Bilbao et al. (1989a) reported a general consensus among different authors classifying cellulose decomposition as endothermic and hemicellulose and lignin decomposition as exothermic.

Analysis of rice straw and rice hulls by DTA (heating rate 5°C min^{-1}) revealed a large exothermic reaction taking place between 300 and 600°C in an inert atmosphere, whereas in air a similar reaction was noted between 280 and 520°C (Nassar,1985). A strong endothermic reaction was also apparent at around 420°C. The first exothermic peak was attributed to the oxidation of volatile products and the second exothermic peak to the oxidation of the charred residue whereas the endothermic peak was explained as the formation and evaporation of volatile products. Four straw varieties subjected to DTA in an oxidising atmosphere and 20°C min^{1} heating programme demonstrated two strong overlapping exothermic reactions occurring within the 250 to 500°C temperature range (Ergudenler and Ghaly,1992). Similarly, peats analysed by either DTA or DSC in oxygen containing atmospheres often give thermal curves comprising of two exothermic peaks in the 300 to 450°C region (Bergner and Albano,1993).

Rice straw and its components were analysed using the more quantitative DSC technique in air and nitrogen atmospheres with a heating rate of 10°C min^{-1} (Lipska-Quinn et al.,1985). In nitrogen, cellulose decomposed endothermically at 340°C whereas in air, two exotherms were seen commencing at 310 and 425°C. Curves for rice straw showed two exothermic reactions taking place in both atmospheres, however the peaks appeared at different

temperatures than with pure cellulose..

Mushroom compost and wheat straw was assessed using DSC at a heating rate of 20°C min^{-1} in an air atmosphere (Sharma,1990) and exothermic reactions were noticed at 325, 400 and 503°C for the compost and 325, 415 and 460°C for the straw. Comparable samples of the compost and straw which had undergone fungal degradation prior to DSC analysis and revealed that resultant thermal curves differed from those of the virgin materials. It was thus considered that the reduced thermal peaks could be accounted for semi-quantitatively due to the degradation of the polysaccharides and lignin by the fungal organisms present. Structural analysis of fungally degraded wood has also been undertaken using DSC with a heating rate of 10°C min^{-1} in air (Reh et al.,1986). Thermal curves were obtained for samples of cellulose, hemicellulose and lignin which were used for reference purposes, as well as for four species of sound wood and corresponding samples of fungally degraded wood. Sound hardwood DSC curves were seen to be the same qualitatively, with quantitative differences being detected. Using data from the decomposition of the reference samples, peaks obtained from sound birchwood thermal degradation at 300, 350 and 475°C were assigned to hemicellulose, cellulose and lignin decomposition respectively (Reh et al.,1986). Lignin, having a high energy content, was thought to be responsible for the most prominent peak seen in the thermal curves of the wood samples, with the area under a peak being related to the amount of the lignocellulosic constituent present in the wood and its heat of combustion. These workers also postulated that different methods of lignin preparation produced different DSC traces due to "preparation mediated artefacts". Modifications

observed in the thermal curves (temperature of peak maxima, peak height and area) of the wood samples exposed to fungal attack were seen to reflect, in a predictable manner, the changes that take place particularly with regard to lignin during the fungal biodegradation of wood. An earlier study by Blackenhorn (1980) also found DSC curves of sound wood and fungally degraded wood to be very different, enabling the workers to detect fungal damage but they could not categorise the fungal organism responsible.

Campanella et al. (1991) applied the DSC method to the analysis of fresh, ancient and waterlogged wood specimens in air at a heating rate of 5°C min^{-1}. An exothermic peak at around 320°C was reported for both fresh and ancient wood degradation and a second exothermic peak was noticed at 460 and 410°C for the fresh and ancient woods respectively. The former peak was attributed to cellulose degradation and the latter to lignin combustion. Interestingly, variations between the DSC traces of these woods were of sufficient magnitude to lead the authors to suggest that DSC may helpful in dating wood samples due to the relationship between the cellulose and lignin contents of wood and its age.

Leaf and needle litter physico-chemical composition and biological degradation over time has also been characterised using DSC (and TG) equipment (Reh et al.,1990). Estimates of the quantity of evolved heat, calculated from DSC peaks and assigned to holocellulose and lignin decomposition, varied as the litter aged. It was speculated that a crystalline form of cellulose may accumulate in decomposing litter and be responsible for the greater heat release from the first reaction which was assumed to be the breakdown of holocellulose.

A selection of coffee varieties subjected to DSC analysis gave somewhat different DSC traces in different atmospheres, nevertheless characteristic and reproducible curves were produced for each coffee variety (Curini et al.,1991).

Studies of foods using DSC have largely focussed on the thermal events associated with high moisture samples at relatively low temperatures, often below 100°C, as opposed to dried samples such as feedstuffs that commence decomposition at much higher temperatures. The fact however remains that DSC has enabled food scientists to gain further understanding of the chemical and molecular changes that occur in complex food systems. It might therefore be possible to enhance our knowledge of the breakdown mechanisms of the lignocellulosic components of forages. Although no evidence could be found in the literature for the application of the DSC technique to forage evaluation, the information DSC has provided pertaining to the lignocellulosic content of wood and straw samples particularly, suggests that this approach merits further investigation.

2.4.3 TG and DSC in the quantitative prediction of chemical and physical constituents in heterogenous biological materials

Thermal analysis methods (TG and DSC) have been assessed with regard to their potential for providing quantitative determinations of chemical and physical constituents in peats (Persson et al.,1986; Bergner and Albano,1993). No reports of similar studies carried out on other biological materials could be found.

Quantitative analysis of the thermal curves of heterogenous materials is not straightforward because the chemical composition of such materials tends to affect the shape of the curve, thus any mathematical method used to evaluate these curves should somehow be based on the actual curve shape itself (Persson et al.,1986). Multivariate statistical methods using separate calibration and validation sample sets have been applied to this type of problem. Persson et al. (1986) obtained results from the TG analysis of 78 peat samples (28 for calibration and 50 for prediction) in a nitrogen atmosphere and proceeded to predict the calorific value, carbon content, hydrolyzable substance and volume weight. Stepwise multiple regression and partial least squares regression (PLSR) methods were compared and achieved a very similar degree of prediction accuracy when tested using the peat calorific value parameter. A calibration model developed for all four properties at the same time produced encouraging results, with standard errors of prediction (SEP) of 0.36MJ kg^{-1} for the calorific value, 0.81% for the carbon content, 3.39% for the hydrolyzable content and 0.05 g ml^{-1} for volume weight.

Simultaneous TG and DSC analysis data from 115 peat samples heated in air were used to estimate 15 different chemical and physical constituents of peats using PLSR in the study of Bergner and Albano (1993). They compared the simultaneous TG/DSC technique with NIRS for 41 of the samples. Both procedures achieved similar results, with estimations of the ash content and Klason lignin proving to be superior with the combination TG/DSC analysis rather than NIRS (SEP=1.8 versus 3.0 and 5.8 versus 7.8 for ash and lignin respectively). In this study, PLSR models using simultaneous TG/DSC values demonstrated

a 25% improvement in parameter predictability over TG and DSC evaluated within separate models.

The successful application of TG and DSC to the prediction of chemical and physical constituents in peat provide very strong justification for exploring the usefulness of these techniques to predict the physico-chemical composition of forages and ruminant animal related forage quality attributes.

3.0 DIFFERENTIAL SCANNING CALORIMETRY TECHNIQUES FOR PREDICTING THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF HAYS

3.1 Introduction

The ability to predict forage quality, particularly with regard to its useful energy content and the amount of the feed that the animal will consume voluntarily, is fundamental to any forage evaluation program. Forages used in animal production systems are extremely variable in terms of their chemical composition, digestibility, and intake potential and it is these factors that determine their nutritional and economic value.

Neutral detergent fibre (NDF) analysis is commonly used to estimate the voluntary intake of forages, although reports by Reid et al. (1988) and Mathison (1990) demonstrated a poor relationship between intake and NDF for a range of forages (R^2 = 0.05 to 0.33 and 0.06 to 0.24 respectively). Similarly, in a review by Minson (1990) r^2 values relating NDF and intake ranged from 0.14 to 0.81. Harlan et al. (1991) reported r^2 values of 0.09 to 0.46 between NDF and dry matter intake (DMI) per 100kg body weight in non-lactating dairy cattle.

Predictions of the available energy content of forages have traditionally related the fibre fraction of the forage to its digestibility. Studies have shown that acid detergent fibre (ADF) is not an accurate predictor of dry matter digestibility (DMD) (Reid et al.,1988), organic matter digestibility (OMD) (Aufrere and Michalet-Doreau.,1988) or digestible energy (DE) content of forages (Mathison,1990). In addition, the modified acid detergent fibre (MADF) technique cannot adequately predict silage OMD (Givens et al.,1989, Barber et al.,1990).

Abrams (1988) observed that 0.68 of the variance in digestible dry matter (DDM) prediction equations using ADF was due to the weak relationship between ADF and DDM and Weiss (1993) considered that the prediction of the available energy content of forages from either ADF or NDF to lack precision.

Near infrared reflectance spectroscopy (NIRS) has proven itself as an economical and useful procedure for estimating forage quality parameters (Norris et al.,1976, Coelho et al.,1988, Baker et al.,1994). Unfortunately it is difficult to relate NIRS results to physical and chemical attributes of forages, thus NIRS is unlikely to increase our understanding of the factors that influence forage quality. Beever (1993), commenting on the prediction of feed intake using methods such as NIRS, outlined the need for approaches which "describe the underlying principles" and of the desire to replace the "less than adequate empirical approach where relationships are casual not causal". There remains therefore, a need to improve our ability to predict forage intake and digestibility.

Differential scanning calorimetry (DSC) is a technique that records the amount of energy that must either be supplied to or withdrawn from a sample in order to maintain a zero temperature differential between the sample and a thermally inert reference material whilst the sample and reference are heated according to a pre-determined temperature programme (Daniels,1973).The rate of energy absorption by the sample is proportional to its specific heat, thus any transition occurring during heating resulting in a change in specific heat is recorded as an endothermic or an exothermic enthalpy change.

Applications of DSC have included the analysis of fuels, clays and minerals, organic and inorganic materials, pharmaceuticals and biological materials (Wendlandt,1986). Researchers

in food science have used DSC extensively to study the physical transitions that take place in foods during heating, with the processes of starch gelatinisation and protein denaturation having received considerable attention (Lund;1983; Biliaderis;1983). White et al. (1990) postulated that inter- and intra-populational differences in the thermal properties of maize starch, measured using DSC, represented differences in the composition and degree of crystallinity of these starches. Other workers have also used DSC in their investigations of crystallisation reactions involving cellulose (Hatakeyama et al.,1976; Kunihisa and Ogawa,1985;1988).

Rice straw and its lignocellulosic components have also been assessed using DSC (Lipska-Quinn;1985). In addition, the DSC technique has been used to examine the biological degradation of wood (Blackenhorn,1980; Reh et al.,1986; Campanella et al.,1991), leaf and needle litter (Reh et al.,1990), and mushroom compost and wheat straw (Sharma,1990). Most of these studies attributed the measured differences between the virgin and partially degraded materials to semi-quantitative changes in the cellulose, hemicellulose and lignin components caused by fungal organisms. Quantitative analysis of 15 chemical and physical constituents found in peat samples has been achieved using DSC, in combination with thermogravimetric analysis, to the same degree of accuracy as with NIRS (Bergner and Albano,1993).

The ability of the DSC technique to characterise crystallinity reactions in high moisture systems and differences in the lignocellulosic complex of woods, straws and peats in low moisture conditions, strongly suggests that this is a technique that merits further investigation with regard to its potential for evaluating forages. The purpose of this work is

to determine if DSC can be used to provide an accurate estimate of forage nutritive value.

3.2 Materials and Methods

Ninety three samples of grass, legume and legume/grass hays harvested from different locations at different stages of maturity between 1980 and 1987 were examined in this experiment. The sample set covered a broad range of forage species including alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), bromegrass (*Bromus inermis*), timothy (*Phleum pratense*), reed canarygrass (*Phalaris arundinacea*), meadow foxtail (*Alopecurus pratensis*) and various combinations of these forage types. These whole plant forage samples were obtained from previous experiments conducted over a seven year period in which digestibilities, at the maintenance feeding level, were measured by total collection procedures using yearling Hereford steers. Further details pertaining to the methodology used for this analysis have been reported previously by Redshaw et al. (1986).

Samples of these forages had subsequently been stored at temperatures of less than 5°C in sealed containers, prior to analysis using the DSC equipment. In this investigation, the samples that had previously been ground through a laboratory hammer mill with a 1mm screen (Christy Hunt Engineering Ltd.,UK.) were reground with a Varimix III mill (Dentsply International Inc.,Milford,DE.) for a total of 100 seconds, in five periods lasting 20 seconds each, to minimize temperature increases within the sample. Moisture contents of the previously dried and stored feed samples were determined by oven drying at 110°C for four hours. The 93 samples were then divided into a calibration sample set of 56 samples and an independent prediction sample set comprising 37 samples. Samples were allocated to each

group randomly within forage type to ensure that an appropriate, relative number of each of the diverse range of forages appeared in both data sets and thus were representative of the total population.

All DSC measurements were made with a 990 Thermal Analyzer with a 910 DSC attachment (Du Pont Instruments, Mississauga, ON.). Calibration of the instrument was carried out according to Du Pont's instructions by measuring the temperature and enthalpy of fusion of pure samples of ice, indium, tin and zinc. For the DSC analysis, 5mg samples were used. Forage samples were analysed in duplicate by heating in the DSC furnace in a static air atmosphere at a rate of 5°C min^{-1}. An oxidising atmosphere of ambient air was chosen over an inert one such as nitrogen in this study because preliminary runs using both atmospheres suggested that an air atmosphere would produce curves with more distinctive differences. Thermal decomposition data for the forage samples were collected over the temperature range 190 to 510°C and recorded on computer disk using a data acquisition system from where it was transferred into a conventional spreadsheet programme for data evaluation. Each run took 64 minutes to complete, with approximately 780 data points recorded, equating to around 12 readings min^{-1} or approximately 2.4 readings per° C. In order to combine data from duplicate samples into one file, temperature readings were rounded to the nearest whole number before averaging mW values from duplicate samples at that temperature.

Two distinct methods of statistical analysis were employed in this experiment. These were stepwise multiple regression (SAS,Institute Inc.,1992) and modified partial least squares regression (PLSR) using NIRS software (NIRS-3; Infrasoft International, Port

Matilda, PA.). For the forward stepwise multiple regression, each of the 17 variables representing chemical composition and animal performance parameters were, in turn, regressed against a data set consisting of the actual mW reading from the DSC curve for each temperature at 5°C intervals with addenda of 1) differences in mW between successive 5°C readings, 2) enthalpy values calculated from the total area under the curve, and 3) enthalpy values between 190 and 340°C, 340 and 420°C, and 420 and 510°C. Bergner and Albano (1993) in their similar study of peat samples demonstrated that reducing the number of points in the data set in this manner had no impact on prediction accuracy. A maximum of three terms was used in each multiple regression equation and only terms with a probability of significance of less than 0.15 were permitted in the equation. Resultant equations developed on the calibration set were then used to estimate each of the 17 forage parameters for the prediction set. Values estimated for the prediction set were subsequently regressed against the actual values in this set. Calibration and validation statistics for the forages are presented in terms of root mean square error (RMSE), co-efficient of determination (R^2) and standard error of prediction (SEP).

The PLSR procedure incorporates the shape of the entire curve into the model rather than just selected points (Persson et al.,1986).A small number of new variables are created from linear combinations of the spectral data and weighted by their co-variance with the reference data (de Boever et al.,1995). Shenk and Westerhaus (1991) have recommended a modified PLRS method for use in NIRS analysis. Data from the DSC curves was analysed using NIRS software (NIRS-3; Infrasoft International, Port Matilda, PA.) and incorporating the log 1/R function (where R in this case represents the mW reading at 5°C intervals), after importation

of the data using additional software provided by the company.

3.3 Results and Discussion

Table 3.1 shows the range of previously determined forage chemical composition and animal related data for the two data sets used separately for calibration and prediction purposes. It is evident that the forages examined covered a broad range in terms of their composition and nutritive value. More importantly, mean values for each of the forage parameters were very similar in both sample sets, with the greatest difference between the calibration and prediction sets for any parameter being in the order of 0.04 of mean value.

Since forage chemical composition data is often used to predict nutritive value, simple and multiple stepwise regression relationships between feed chemical constituents and animal intakes and digestibilities are reported in Table 3.2. Interestingly, although NDF is commonly used as a predictor of intake, it did not feature in the regression equations developed for dry matter intake (DMI). Indeed, CP demonstrated the strongest relationship with DMI, giving an R^2 value of 0.30, but even this lies at the bottom of the range of values relating CP to voluntary intake tabulated by Minson (1990). Even with both ADF and CP in the equations, calibration and prediction R^2 values only reached 0.45 and 0.22 respectively. Comparable values for digestible energy intake (DEI) achieved just a slight improvement at 0.56 and 0.41 for the calibration and prediction set respectively. As expected, ADF proved to be the best single indicator of dry matter digestibility (DMD) accounting for 34% of the variance in the calibration samples. This also lies within the 0.23 to 0.93 range reported in a review by Minson (1982) and the 0.0 to 0.45 range reported by Reid et al. (1988). When

GE and lignin also entered the equation, the calibration R^2 increased to 0.45 and the prediction R^2 was 0.58. In almost all cases, predictions of cattle digestibility and intake parameters using multiple regressions were better than for the original simple regressions, using a single measured constituent, for each parameter in the calibration set.

The best single predictor of CP was the ash content, which explained 51% of the measured variation. Overall the ability of ash, ADF and acid detergent insoluble nitrogen (ADIN) to account for CP variability was reasonable at 67% and 53 % for the calibration and prediction sets respectively. Estimations of NDF, ADF, hemicellulose and lignin from combinations of chemical constituent data were, as one might anticipate, attained with a high degree of accuracy due to the inherent relationships between the variables.

The application of the multivariate approach to the prediction of the nutritive value of forages from combinations of feed chemical constituent analyses, using separate calibration and validation data sets has not to our knowledge been used before. This approach produced encouraging results for multiple regression equations, suggesting that the use of such equations may improve predictions above that achieved if only a single food constituent is used for preditive purposes.

Examples of duplicate DSC curves are shown in Figures 3.1 and 3.2. Data were collected only in the 190-510°C temperature range because thermal decomposition reactions were confined within these temperatures. Below 190°C almost no detectable reaction activity took place, with the exception possibly of the endothermic loss of moisture, although this was not significant, given that sample dry matters were 91.7% ± 0.77. The greatest divergence between duplicate samples was consistently seen to occur during the final peak on the DSC

curve at around 430 to 470°C. Differences between ten sets of duplicate curves, selected at random, are given in Table 3.3. Average differences at the specific temperatures for the entire curve ranged from 0.72 to 2.30 mW for the ten curves, which equate to between 1.4% and 4.7% of the overall average mW readings. The maximum recorded differences at specific temperatures were relatively large (3.7% to 10.2% of the average of these two values), however these occurred exclusively in a narrow temperature range during the final peak (430-470°C). From these results, it would appear that duplicate runs are adequate for the majority of samples. If the accuracy of the DSC readings during the final peak is particularly important, duplicate runs are probably insufficient, and additional runs for this section of the curve would be necessary.

In Figure 3.3, four example DSC curves (duplicates combined) are presented to demonstrate the range of DSC curves produced from the forage samples tested. Clearly, the DSC curves shown differ markedly from one another, some samples having three discernable reaction zones and others just two. A tendency was, however, noticed for many of the samples within each forage type to produce a similar shaped DSC curve.

Forage samples gave rise to DSC curves whose general shape clearly resembles that of curves reported in the literature for DSC analysis of wood samples (Reh et al.,1986; Campanella et al.,1991), peat samples (Bergner and Albano,1993) and wheat straw samples (Sharma 1990) and resemble curves of leaf and needle litter samples to a lesser extent (Reh et al.,1990). Energy flux values of approximately 3 to 12 mW mg^{-1} in this study were in the same range as those reported by Reh et al. (1986) for wood samples and Bergner and Albano (1993) for peat samples.

The work of Reh et al. (1986) included the analysis of cellulose, xylan (hemicellulose) and three lignin preparations as reference samples, in addition to samples of sound and fungally degraded wood. Although these reference samples were not pure, it was considered that the first peak occurring at 350°C could be attributed to cellulose and hemicellulose degradation and the second at around 475°C to lignin decomposition. Likewise, other workers have apportioned the two main DSC peaks seen in the analysis of lignocellulosic materials, to holocellulose (cellulose and hemicellulose) and lignin degradation respectively (Sharma,1990; Reh et al.,1990; Campanlla et al.,1991). Small peaks at intermediate temperatures (380-440°C), observed from some of the hays in this study were similar to those encountered by Sharma (1990) for undegraded wheat straw, who postulated that they may be explained by hemicelluloses existing in close association with lignin. Consequently it would appear reasonable to assign the two main DSC peaks of hays, which took place at around 310-325°C and 430-470°C to holocellulose and lignin, respectively, and thus consider sample differences in these two peaks to semi-quantitatively represent the relative amounts of these components present. In an attempt to verify this, DSC values at 5°C intervals within the two main peaks (310-325°C and 430-470°C), for all 93 samples, were regressed against the forage holocellulose and lignin contents respectively. The holocellulose content was estimated by subtracting the lignin content from the NDF values assuming that NDF contains the cellulose, hemicellulose and lignin components . Using this approach, r^2 values of approximately 0.4 were obtained for the relationship between holocellulose and the energy flux values from the first peak. No relationship ($r^2 = 0.00$) was found between DSC values from the final peak and the lignin content of the forages. A dry sample of purified bovine

casein (Figure 7.5 in Appendix) examined using DSC under the same experimental conditions suggested that proteins also undergo thermal decomposition at high temperatures, particularly those above 420°C. This may mean that results pertaining to the degradation of lignin in forages are confounded by protein degradation at the same temperatures.

Table 3.4 shows the relationships between DSC curve mW readings plus 5°C changes in DSC readings with feed composition using simple linear and stepwise multiple regression procedures. Relationships were obtained between DSC measurements and all forage parameters for which data were available. Somewhat surprisingly, relationships between DSC and forage GE content were extremely poor, especially given that DSC gives a measure the energy flux occurring within the sample during heating. This was probably because of the small range in the GE content of the samples (17.4-19.6 MJ kg^{-1}). Percentage CP and NDF were the forage parameters most accurately predicted in this experiment, with R^2 values of 0.61 (SEP = 1.91) and 0.72 (SEP = 3.84) respectively. The technique therefore does appear to have some potential usefulness in predicting the NDF and CP content of forages, but the procedure appears to be inferior to NIRS where R^2 values of 0.94-0.98 and 0.79-0.91 have been obtained for CP and NDF respectively, by other workers (Norris et al.,1976; Redshaw et al., 1986; Martin and Linn,1989; Snyman and Joubert,1993). Ash and ADF percentage values demonstrated a significant relationship with certain DSC readings in the calibration set but the strength of this relationship diminished when applied to the prediction set. Lignin percentage (Goering and Van Soest,1970) and ADIN were very poorly estimated using DSC. Admittedly, chemical analysis procedures for lignin are far from ideal. Reh et al. (1986) analysing wood lignins isolated using three different procedures and subjecting

them to DSC, found the resultant curves to be remarkably dissimilar and attributed these differences to "preparation mediated artefacts" peculiar to each lignin extraction procedure.

Dry matter intake and DEI could not be predicted accurately from DSC results, with R^2 values for the calibration set of 0.60 and 0.57 respectively, only being translated into values of 0.13 and 0.24 respectively for the prediction samples (Table 3.4). Redshaw et al. (1986) using NIRS to estimate DMI and DEI and utilising a data set with many actual samples common to this study, found that 72% and 78% of the variance in DMI and DEI respectively, was accounted for. Dry matter digestibilities (R^2 = 0.13) and energy digestibilities (R^2 = 0.11) were not adequately predicted using DSC. Corresponding validation set R^2 values achieved elsewhere using NIRS were 0.65 (Norris et al.,1976), and 0.68 (Redshaw et al.,1986) for the DMD of a range of forages and 0.76 (Barber et al.,1990) and 0.77 (Baker et al.,1994) for the OMD of grass silages.

The first and second terms that entered the regression equation for DMD and OMD were similar to those entered for ADF, but no such similarity was present for DMI or DEI, and NDF. This contrasts somewhat with the findings of Norris et al. (1976) using NIRS, who found the best wavelength for DMD and ADF to be the same, and the best wavelength for DMI and DEI to be the same as the second best wavelength for NDF and CP.

Actual temperatures and 5°C changes in temperature that were entered into the prediction equations for each forage parameter were not focussed on any particular section of the curve, rather they included points from a very wide temperature range. Enthalpy values were not related to forage chemical composition or animal related parameters.

Stepwise multiple regression and partial least squares regression (PLSR) have both been

used for NIRS data analysis. Shenk and Westerhaus (1991) reported an 18% improvement in the standard errors of prediction using modified PLSR rather than modified stepwise regression. For this study we also wanted to compare simple and multiple regression relationships, thus all of the nutritive value predictions detailed above were reached using simple linear and multiple stepwise regression. In all cases, the PLSR results were very similar to the stepwise multiple regression results and are presented in the appendix.

In the stepwise multiple regression analysis, a maximum of three terms was allowed in each equation, whereas in the case of NIRS five or more wavelengths have been used for equation development (Norris et al.,1976; Martin and Linn,1989; Barber et al.,1990).The number of terms permitted in a regression is important because the use of too many terms in the calibration set will result in overfitting of the data and sacrifice prediction accuracy (Westerhaus,1989). The inclusion of up to six terms in any equation was attempted here, but although this reduced the root mean square error (RMSE) and increased the R^2 for the calibration set, it did not lead to any substantial change in prediction accuracy.

Bergner and Albano (1993) in their studies using a simultaneous TG/DSC technique for the quantitative analysis of peat samples reported this approach could be used to predict such factors as energy values, elemental composition (eg. carbon, hydrogen, nitrogen), ash and lignin (SEP = 0.6, 1.8, 2.0 and 4.5 for energy (MJ kg^{-1}) , carbon (%), ash (%) and lignin (%) respectively). These workers used PLSR in the analysis of their data, but the data from their 115 samples underwent statistical analysis using an internal statistical validation procedure only. Although this approach is deemed acceptable for preliminary calibration equation development (Windham et al.,1989) and has been used for NIRS equation validation when

insufficient samples were available for a separate validation to be carried out (de Boever et al.,1995), it has been stressed that calibration equations should be tested against an independent sample set to obtain an independent measure of equation accuracy. (Murray,1986; Westerhaus,1989; Windham et al.,1989; Barber et al.,1990). The results from our experiment confirm the necessity of an independent validation procedure. The suggestions of Bergner and Albano (1993) of the accurate evaluation of peat by thermal analysis may therefore have been rather optimistic.

In conclusion, some significant improvements in the accuracy of forage quality estimations from existing databases may be possible by using combinations of chemical analysis data to develop multiple regression equations which can then be independently validated. The DSC technique was evaluated using the same calibration and validation approach that is routinely used for predicting forage quality in NIRS analysis. Unfortunately, this technique, which provides a whole spectrum of physico-chemical information on the thermal decomposition of lignocellulosic materials, was not closely related to results from chemical analyses, nor could it provide a better estimate of forage digestibility or intake potential than NIRS or chemical analysis. Further empirical investigations using DSC to evaluate forages are therefore unwarranted unless evidence of a more direct molecular relationship between DSC measurements and the composition of plant materials is forthcoming.

Table 3.1. Characteristics of alfalfa and grass hay feed sample dry matter.[a]

	Calibration[c]			Prediction[d]		
Item[b]	Mean	SD[e]	Range	Mean	SD	Range
GE (MJ kg^{-1})	18.4	0.42	17.4-19.4	18.6	0.42	17.9-19.6
CP (%)	13.4	4.01	5.6-20.8	13	3.19	6.0-18.7
NDF (%)	57.6	11.0	39.2-77.0	60.3	9.41	41.3-77.1
ADF (%)	39.9	5.71	28.8-56.6	41.8	5.75	31.1-57.4
Hem. (%)	17.8	7.98	3.9-34.8	18.5	7.28	6.5-33.0
Lignin (%)	7.3	2.04	4.2-12.3	7.46	2.06	4.1-11.5
Ash (%)	8.0	2.26	4.1-10.5	7.2	2.47	4.2-10.4
ADIN (%)	0.21	0.09	0.06-0.46	0.22	0.10	0.07-0.44
DMI (g $kg^{-0.75}$)	101.6	17.9	70-132	98.3	15.8	57-124
DEI (kJ $kg^{-0.75}$)	1098	238	699-1699	1048	217	498-1402
Digestibility (%)						
DM	59.9	3.59	48.0-67.0	58.0	4.71	44.6-68.5
OM	61.6	3.86	49.6-69.0	59.7	4.71	46.7-69.8
GE	58.3	4.21	45.7-66.8	57.0	4.92	43.8-67.4
CP	62.4	10.3	41.0-77.5	60.8	10.8	34.4-78.1
NDF	52.9	9.41	26.8-77.3	53.0	7.37	39.0-68.2
ADF	48.4	6.97	26.8-66.3	47.8	5.05	37.9-62.2
DE (MJ kg^{-1})	10.7	0.88	8.5-12.8	10.6	0.96	8.5-12.9

[a]In the experiment 93 hays were examined..
[b]Abbreviations used: ADF=acid detergent fibre, ADIN=acid detergent insoluble nitrogen CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, NDF=neutral detergent fibre, and OM=organic matter.
[c]Calibration set contained 56 samples.
[d]Prediction set contained 37 samples
[e]Standard deviation.

Table 3.2. Simple and multiple regression relationships between feed chemical composition and nutritive value[a]

		Calibration				Prediction[f]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[e]	r^2	RMSE	R^2	SEP[g]	R^2
CP (%)	Ash, ADF, ADIN	2.84	0.51	2.35	0.67	2.11	0.53
NDF (%)	Ash, Lignin, ADF	7.17	0.58	3.83	0.89	3.82	0.83
ADF (%)	NDF, GE, Lignin	4.02	0.51	1.76	0.91	1.85	0.89
HEM (%)	NDF, GE, Lignin	4.01	0.75	1.76	0.95	1.86	0.93
Lignin (%)	ADIN, ADF, HEM	1.40	0.50	0.81	0.83	0.85	0.81
Ash (%)	NDF, CP	1.32	0.60	1.19	0.67	1.01	0.48
ADIN (%)	Lignin, CP, NDF	0.06	0.50	0.05	0.67	0.06	0.45
DMI (g $kg^{-0.75}$)	CP, ADF	14.6	0.30	2.74	0.45	1.74	0.22
DEI (kJ $kg^{-0.75}$)	CP, GE, ADF	47.7	0.30	39.0	0.56	29.3	0.41
Digestibility (%)							
DM	ADF, GE, Lignin	2.95	0.34	2.74	0.45	1.74	0.58
OM	ADF, GE, Lignin	3.08	0.37	2.88	0.47	1.79	0.63
GE	ADF, GE, Lignin	3.32	0.39	3.02	0.51	2.12	0.57
CP	CP, ADIN, HEM	6.30	0.63	5.04	0.77	3.07	0.82
NDF	HEM, CP, NDF	6.10	0.59	5.42	0.68	4.34	0.63
ADF	NDF, CP, Lignin	5.81	0.32	5.29	0.45	3.56	0.27
DE (MJ kg^{-1})	ADF, GE, Lignin	0.18	0.30	0.13	0.61	0.11	0.55

[a]In the experiment, 93 hays were examined.
[b]Abbreviations used: ADF=acid detergent fibre, ADIN=acid detergent insoluble nitrogen, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to allow no more than three variables in equation.
[e]Root mean square error.
[f]Predictions based on multiple regression equations developed for the calibration set.
[g]Standard error of prediction.

Table 3.3. Differences between ten randomly selected duplicate DSC curves

Sample No.	Mean mW[b]	Differences (mW)[a]			Differences (%)	
		Mean	SD[c]	Maximum	Mean[d]	Maximum[e]
8224	50.6	1.36	1.64	6.93	2.69	5.97
8227	51.0	1.63	1.13	5.96	3.20	7.66
8507	48.7	2.30	1.82	12.9	4.72	9.27
8219	49.8	0.72	0.65	3.45	1.44	7.09
8416	49.2	1.37	1.12	5.84	2.79	10.2
8325	53.1	1.97	3.31	23.4	3.71	12.4
8018	46.7	1.01	0.79	2.80	2.15	3.73
8715	52.4	1.15	1.51	6.22	2.06	4.65
8317	52.3	1.56	1.14	5.25	2.98	6.72
8401	50.2	1.39	1.78	11.6	2.78	9.50

[a]Differences in mW between duplicate curves.
[b]Overall mean of mW values from the DSC curve.
[c]Standard deviation of the differences.
[d]Mean difference (mW) expressed as a percentage of overall mean (mW).
[e]Maximum difference between curves, expressed as a percentage of the mean of the curves at that specific temperature.

Table 3.4. Simple and multiple regression relationships between feed differential scanning calorimetry measurements (5°C min^{-1} heating rate) including 5°C temperature changes and feed composition and nutritive value using stepwise multiple regressions[a]

		Calibration				Prediction[e]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[f]	r^2	RMSE	R^2	SEP[g]	R^2
GE (MJ kg^{-1})	220	0.10	0.10	-	-	0.04	0.02
CP (%)	380, 500, 270	2.40	0.65	1.95	0.78	1.91	0.61
NDF (%)	370, 210,Δ270	5.96	0.71	4.22	0.86	3.84	0.72
ADF (%)	475,Δ210,Δ465	4.15	0.48	3.54	0.64	3.37	0.24
HEM (%)	Δ370,Δ310,Δ430	4.61	0.67	3.87	0.78	4.25	0.33
Lignin (%)	440,330,Δ210	1.78	0.16	1.58	0.37	1.01	0.01
Ash (%)	390,260,Δ390	1.14	0.69	1.08	0.73	1.19	0.46
ADIN (%)	Δ210,Δ235,Δ430	0.08	0.17	0.07	0.42	0.04	0.20
DMI (g $kg^{-0.75}$)	320,Δ390,Δ445	13.0	0.48	11.7	0.60	12.4	0.13
DEI (kJ $kg^{-0.75}$)	320,465,Δ445	43.4	0.43	38.2	0.57	32.4	0.24
Digestibility (%)							
DM	465,Δ205,Δ235	3.22	0.21	3.00	0.34	2.03	0.13
OM	470,Δ205,Δ290	3.37	0.25	3.01	0.43	3.01	0.10
GE	470,Δ340,Δ395	3.63	0.27	3.23	0.44	3.33	0.11
CP	365, 495,Δ200	6.73	0.58	5.47	0.73	4.13	0.56
NDF	Δ285,200,Δ325	7.71	0.34	6.83	0.50	4.90	0.40
ADF	Δ285,Δ295,Δ330	5.99	0.27	5.58	0.39	4.13	0.43
DE (MJ kg^{-1})	470,Δ340,Δ395	0.18	0.24	0.17	0.37	0.15	0.08

[a]In this experiment 93 hays were examined.
[b]Abbreviations used: ADF=acid detergent fibre, ADIN=acid detergent insoluble nitrogen, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]Components comprise actual temperatures or the 5°C change in temperature recorded at this temperature. First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to no more than three variables in equation. Missing values signify that no multiple regression equation was developed.
[e]Based on multiple regression equation developed for the calibration set.
[f]Root mean square error.
[g]Standard error of prediction.

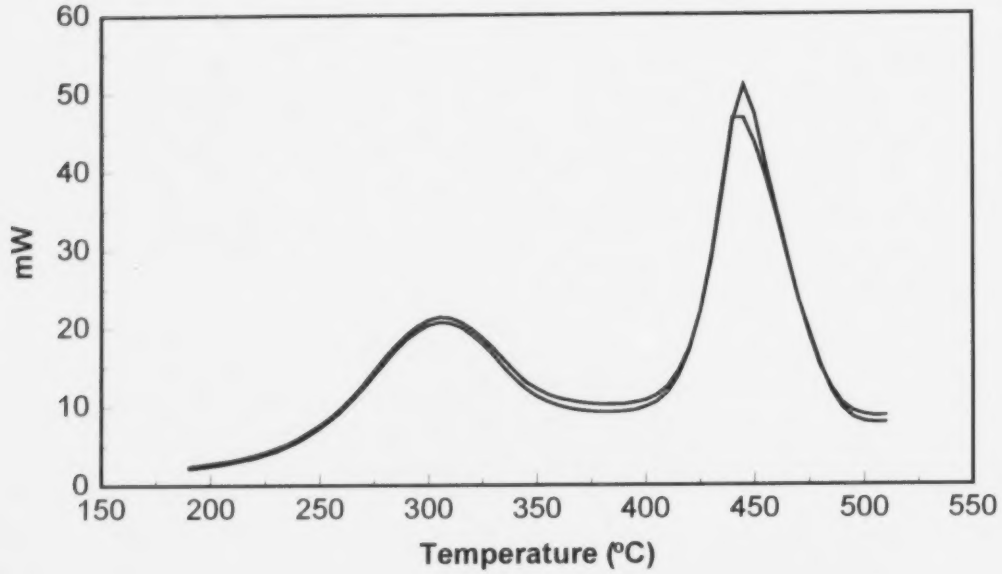

Figure 3.1. Duplicate DSC curves of alfalfa sample 8507 (5.0 mg; 90.3% DM).

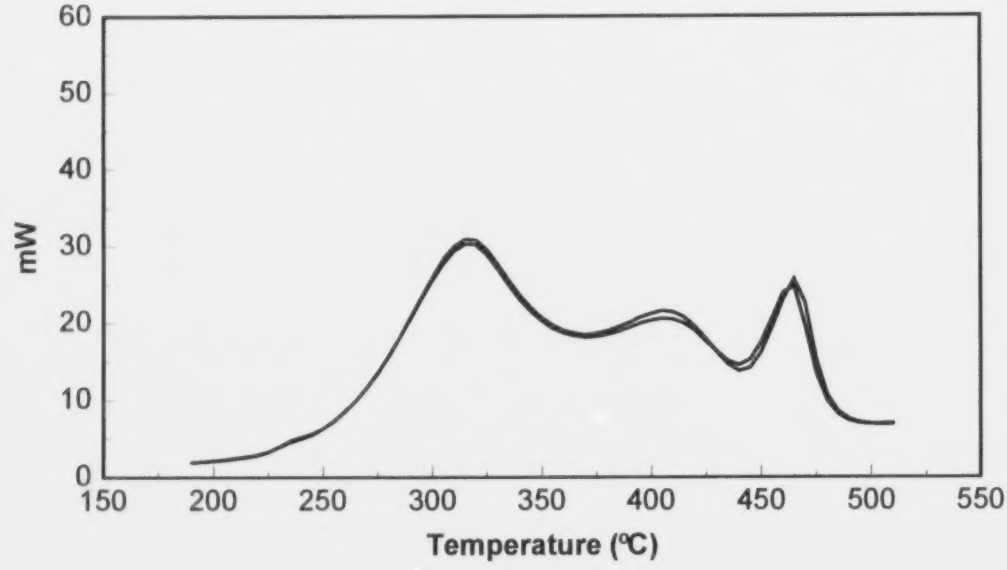

Figure 3.2. Duplicate DSC curves of timothy sample 8226 (5.0 mg; 91.9% DM).

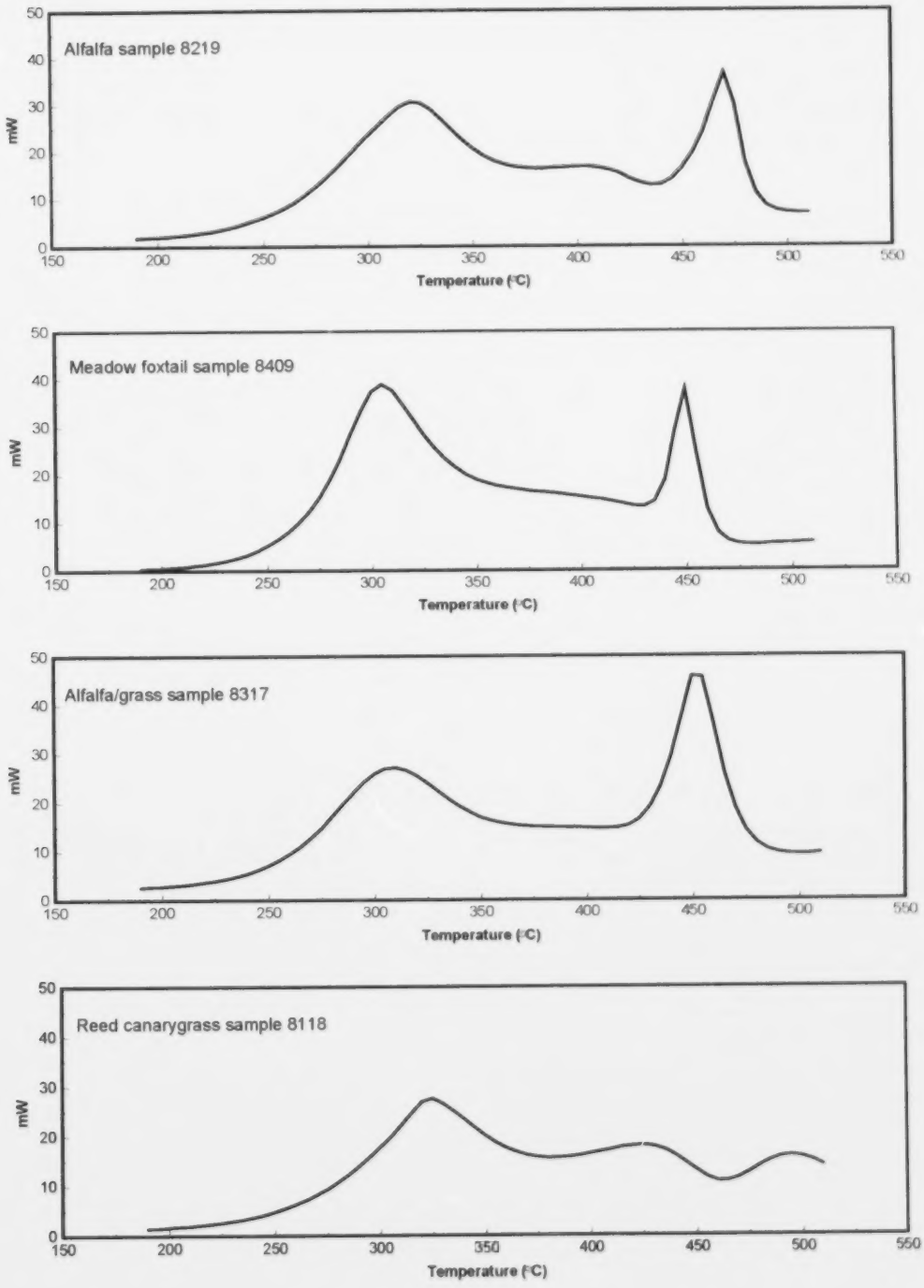

Figure 3.3. Four example DSC curves of hay samples (5.0 mg sample weight; 90.8- 92.0% DM).

4.0 THERMOGRAVIMETRIC TECHNIQUES FOR PREDICTING THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF HAYS

4.1 Introduction

The economic and nutritive value of a forage is primarily determined by the quantity of useful energy it contains and the amount of the forage the animal will voluntarily consume. As discussed in the previous chapter, the conventional methods of estimating forage digestibility and voluntary intake, ADF and NDF respectively, have provided variable and often inadequate results.

Multivariate analysis methods, like NIRS provide us with some sense of the molecular environment present in foods in a direct manner (Murray,1988) and provide more information pertaining to the sample than is the case with traditional chemical analyses (Coleman and Murray,1993). Indeed, NIRS is now widely recommended for the routine evaluation of forages (Redshaw et al.,1986; Barber et al.,1990; Baker et al.,1994).Coleman and Murray (1993) postulated that the analysis of faecal material, in addition to hay samples, using NIRS, may enhance our understanding of forage quality attributes. Nevertheless, NIRS has been criticised for not specifically being able to relate the physico-chemical properties of forages to forage quality attributes (Van Soest,1982).

The thermal analysis technique of differential scanning calorimetry (DSC) has been assessed regarding its potential in evaluating forages using the multivariate approach (Chapter 3). Despite the association between DSC curves of lignocellulosic materials and the respective quantities of their main individual components (Sharma,1990; Reh et al.,1990; Campanella et al.,1991), DSC could not accurately account for sample differences in

measured forage parameters as discussed in the previous chapter.

Thermogravimetric (TG) analysis is a technique whereby the weight of a substance is recorded as a function of temperature or time as a sample is heated or cooled under defined conditions (Keattch and Dollimore,1975, Earnest,1988). The technique has been used to analyse fuels, clays, inorganic compounds, polymers and pharmaceutical products (Wendlandt,1986) and in studies looking at the structure and chemical composition of foodstuffs such as cereal, meat and dairy products (Ma and Harwalkar,1991). Thermogravimetry has been used to establish the dry matter, volatiles, fixed carbon and ash contents of coals and related products (Charsley and Warrington,1988; Weidemann,1988).

Bilbao et al. (1989a) stated that the physical and chemical nature of lignocellulosic materials markedly affects the rate at which they lose weight during thermal decomposition. Experimental determinations relating to the reaction kinetics of lignocellulosic materials using TG have been carried out on cellulose (Bilbao et al.,1987), hemicellulose and lignin (Bilbao et al.,1989a), cereal straw (Bining et al.,1987, Bilbao et al.,1989b; Sharma,1990; Ergudenler and Ghaly,1992), rice straw and hulls (Ebeling and Jenkins,1987; Nassar,1985; Lipska-Quinn,1985), and various lignocellulosic materials (Bilbao et al.,1989b; Reh et al.,1990; Campanalla et al.,1991; Balci et al.,1993 Orfao and Figuieredo, 1993). The contribution of each of the components (cellulose, hemicellulose and lignin), to the pyrolysis rate of biomass is proportional to its presence in the material concerned (Koufapanos et al.,1989). The weighted contributions of hemicellulose, cellulose and lignin have been shown to satisfactorily predict the pyrolysis of pinewood and barley straw (Bilbao et al.,1989b). Moreover, TG techniques have also been used to predict physical properties and chemical

constituents in peat samples (Persson et al.,1986; Bergner and Albano,1993). Simultaneous TG/DSC analysis of peat samples has achieved the same degree of accuracy in predicting chemical and physical parameters, such as energy, ash, carbon, glucose and lignin contents, as that obtained using NIRS (Bergner and Albano,1993). It follows, therefore, that TG analysis may be of value as a predictor of forage quality.

The purpose of this very preliminary investigation, using a limited number of forage samples, was to explore the possibilities of using TG to analyse both feed and faecal samples in order to gain an estimate of forage quality.

4.2 Materials and Methods

Eighteen samples of hay, seven alfalfa (*Medicago sativa*), four timothy (*Phleum pratense*), five bromegrass (*Bromus inermis*) and two orchardgrass (*Dactylis glomerata*), with corresponding faecal material were used in this experiment. These whole plant forage samples were obtained from previous experiments conducted over a four year period in which digestibilities, at the maintenance feeding level, were measured by total collection procedures using yearling Hereford steers. Cutting dates varied from 7 June to 1 Oct for the 14 hays for which this information was available. These forage samples had been stored at temperatures either less than 5°C or at less than 0 C before they were used in this experiment. Further information concerning these samples has been given previously (Redshaw et al.,1986).

Thermogravimetric measurements were obtained on forage samples in two distinct studies. In the first experiment, samples of forage material (mean sample size = 5.41± 0.36

mg; mean ±SD) and faecal material (= 5.84 ±0.54 mg) were separately analysed using a 1090 Thermoanalyser with a model 951 Thermogravimetric analyser attachment (Dupont Instruments, Mississauga, ON). The system was flushed with air at a rate of 20 ml min^{-1} and the temperature of the sample was raised by 10°C min^{-1} from room temperature to 600°C.

In the second experiment, 10 mg samples of the same 18 forages underwent High Resolution™ TG using a Thermogravimetric Analyser, Model 2950 with a High Resolution TG analysis capability (TA Instruments, New Castle, DE.). This technique entails high heating rates in regions where no decomposition rate changes are occurring, but smoothly and continuously adjusts the heating rate in response to changes in sample decomposition. In this instance a maximum heating rate of 50°C min^{-1} from room temperature to 600°C and a nitrogen purge flow rate of 100ml min^{-1} were chosen. Prior to thermal analysis, the samples which had previously been ground through a laboratory hammer mill with a 1 mm screen (Christy Hunt Engineering Ltd., UK.) were subsequently reground using a Varimix III mill (Dentsply International Inc.,Milford, DE.) for a total of 100 sec. in five periods lasting 20 sec. each to minimise rises in temperature.

For both experiments, data was based on one TG run per sample. Weight of DM for use in the analysis was taken as the weight of the sample at 100°C and weights during heating were expressed as a percentage of this weight.

The 18 samples of alfalfa, brome, timothy and orchardgrass were representatively subdivided into a calibration set (12 samples) and a prediction set (six samples). Pearson's correlation coefficients were plotted for each forage parameter in turn for the entire data set. Stepwise multiple regressions (SAS Institute Inc.,1992) were carried out. Each of the 17

variables representing chemical composition and animal performance parameters were, in turn, regressed against a data set consisting of TG residual weights at 10°C intervals with an addendum of residual weight differences between successive 10°C readings. A maximum of three terms was used in each equation and only terms with a probability of significance of less than 0.15 were permitted in the equation. Resultant equations developed on the calibration set were then used to estimate each of the 17 forage parameters for the prediction set. Values estimated for the prediction set were subsequently regressed against the actual values in this set. Variables relating to weight changes at temperatures below 160°C were omitted from any regression equations.

Residual weight data from the high resolution TG experiment were also analysed using near infrared reflectance software (NIRS-3, Infrasoft International,Port Matilda, PA.) and incorporating the log 1/R function (where R in this case represents HRTG residual weights at 10°C intervals) after importation of the data using additional software from the company.

4.3 Results and Discussion

Chemical composition and animal related intake and digestibility data for the alfalfa and grass hay samples, split into a calibration sample set and a separate prediction sample set, are shown in Table 4.1. In Table 4.2, chemical composition data for corresponding faecal samples are reported. The 18 forages examined covered a broad range in terms of their composition and nutritive value. Mean and range values for each parameter were relatively similar across the calibration and prediction data sets, for such a small number of samples.

Simple and multiple regression relationships between feed chemical constituents and

animal intake and digestibility data are shown in Table 4.3. Despite the limited number of samples examined, relationships between the various chemical analysis variables were, as anticipated, significant (prediction set R^2= 0.61-0.99). The terms used in the predictions were similar, although not identical to those calculated using a much larger sample set (Chapter 3). Reasonable accuracy was achieved for the estimations of DMI and DEI in the prediction set (R^2= 0.32 and 0.55 respectively) which was higher than corresponding values in the larger sample set examined in Chapter 3 (0.22 and 0.41). Estimations of DM and OM digestibilities were particularly poor, even though R^2 values of almost 0.90 were found for the calibration samples. In the large sample set in Chapter 3 however, DMD and OMD were predicted relatively accurately (R^2=0.58 and 0.63 respectively). This underlines the fact that if multivariate techniques are to be used, then an adequate number of samples must be included in order to obtain reliable results.

Figures 4.1 and 4.2 contain the high resolution TG (HRTG) curves for the seven alfalfa hays and five bromegrass hays analysed in this study. The general shape of all these TG curves is typical of that for the curves of other lignocellulosic materials including woods (Shafizadeh and McGinnis,1971; Koufopanos et al.,1989), mushroom compost (Sharma,1990), peat (Persson et al.,1986; Bergner and Albano,1993), rice straw (Nassar,1985; Lipska-Quinn et al.,1985), rice hulls (Nassar;1985; Ebeling and Jenkins,1987) and cereal straw (Bining et al.,1987; Sharma,1990; Ghaly and Ergudenler 1991). It is evident that the HRTG curves for these grass and legume hays do not demonstrate any major inter-species differences. The greatest deviation between individual curves is seen to occur in the 225 to 275°C temperature range and at temperatures above 325°C.

Normal TG (NTG) curves of these hays (Figure 4.3) were very similar to HRTG curves, however in the 410 to 440°C temperature range, a sharp decrease in weight was observed for all samples, which was not present in the HRTG curves. When NTG curves from different species of forages were superimposed upon one another, no obvious consistent species differences were discernible. Apparent differences in the thermal curves of the hays from the two experiments, NTG and HRTG, could possibly be explained by the different experimental protocols employed. The NTG analysis was undertaken in an air atmosphere using a 10°C min^{-1} programmed heating rate and the HRTG was conducted in a nitrogen atmosphere using a 50°C min^{-1} maximum heating rate. Other workers have demonstrated that both different atmospheres and heating rates affect the resultant TG curves (Lipska-Quinn,1985; Bining et al.,1987; Ghaly and Ergudenler,1991). It was noted that the residual weights, once the thermal decomposition was complete, were greater for the HRTG than for the NTG. Both nitrogen atmospheres and high heating rates have been associated with increased residual weights (Lipska-Quinn et al.,1985; Bining et al,1987; Ghaly and Ergudenler,1991). Increased residual weights at high heating rates are indicative of insufficient time being available for the decomposition reactions to occur (Bining et al.,1987; Ghaly and Ergudenler,1991). This should not, however have been a significant problem with the HRTG since the rate of heating is slowed down when the reactions are occurring.

Hemicellulose can be considered to be highly decomposed once 260°C is reached and cellulose and lignin commence decomposition at around 230 and 290°C respectively (Bilbao et al.,1989b). However, lignin has also been shown to begin decomposition at lower temperatures than cellulose or hemicellulose and not to decompose as completely as

cellulose or hemicellulose (Shafizadeh,1968; Koufopanos et al.,1993). The large weight losses observed in the 230 to 330°C temperature range are therefore to be expected.

Simple and multiple regression relationships between sample weights at 10°C intervals plus the difference between each sample weight at 10°C intervals, and feed chemical composition and nutritive value data are shown for NTG in Table 4.4 and for HRTG in Table 4.5. A strong relationship (R^2 = 0.67 to 0.97) was established for NTG and HRTG data and all forage chemical parameters (with the exception of NDF for the NTG data) for the calibration sample set. When however, the equations developed from the calibration samples were applied to the six validation samples the results were rather disappointing in terms of estimating the parameters of interest. For the NTG technique, validation set predictions achieved for CP, NDF and ADF were not significant (R^2 = 0.18, 0.32 and 0.33 respectively). With the HRTG procedure, CP, NDF and ADF were seemingly estimated to a higher degree of accuracy in the prediction set (R^2 = 0.72, 0.32 and 0.44). These values estimated from NTG and HRTG data are much worse than comparable ones reported using larger data sets in NIRS studies (Norris et al.,1976; Redshaw et al.,1986; Snyman and Joubert,1991). The reason for the large variation in the CP predictions from the NTG and HRTG data, using the same forages for validation, (R^2=0.18 versus 0.72) is not known and probably not meaningful. Interestingly, TG did not predict the ash content of the sample as well as might be anticipated, given the general similarities between these procedures. Bergner and Albano (1993) reported that thermal analysis techniques estimated the ash content of peat samples more accurately than did NIRS (SEP=1.8 and 3.0 for TG/DSC and NIRS respectively), but in their study an independent validation sample set was not used.

Comparisons of the temperature related terms that entered the equations for each variable in Tables 4.4 and 4.5 indicate that there is very little similarity or consistency between temperatures selected for DSC or TG procedures. Also, a substantial portion of entered terms occurred at the extreme ends of the temperature scale where differences between samples were small. In fact, to help alleviate this problem, temperature related values below 160°C were prevented from entering any equations. The magnitude of the differences in some instances may indicate that terms entering certain equations may have done so in a random fashion. Possible explanations for this could include the fact that simply too few samples were examined or indeed that no stable relationship exists between TG data and many forage quality parameters.

In an attempt to investigate this further, correlation coefficients were calculated for the forage parameter data and TG data at each 10°C temperature increment for all 18 hay samples. Correlations for TG and high resolution TG data were superimposed on the same graph and the resultant relationships with temperature are shown for CP and ash in Figures 4.4 and 4.5 respectively. A common pattern emerges in both graphs, with the HRTG positive correlation data points tending to be skewed towards higher temperatures. There were significant deviations occurring between the two TG techniques at temperatures below 180°C, between 275 and 325°C, and above 400°C. The very poor values for the NTG curves between 410 and 440°C correspond to the sudden weight losses that occurred in this period exclusively with NTG. The differences at the extreme ends of the temperature scale are especially important as these show the strongest correlation and hence are preferentially selected for entry into the regression equations. The reason for the strong negative

relationship at low temperatures for the high resolution TG curves is unclear, but is probably not meaningful as the weight losses and the differences between individual samples during this period are extremely small. Additional graphs of NTG and HRTG correlation co-efficient values at each temperature are given in the appendix.

No significant regression equation could be developed between DMI and NTG data (Table 4.4). Similarly, DMI and DEI predictions based on the validation samples demonstrated no relationship with HRTG data (R^2=0.00 and 0.08 respectively, Table 4.5). Despite the fact that calibration equations developed for DMD using NTG and HRTG data had high R^2 values of around 0.90, when they were applied to the validation samples no relationship was found. Other digestibility predictions were generally poor and inconsistent, for example, hemicellulose digestibility was apparently estimated accurately, but percentage hemicellulose was not for the NTG data (R^2=0.75 and 0.20 respectively). In comparison with DSC, the relationships between NTG and HRTG and particularly the intake and digestibility data, were in most cases either poor or inconsistent. It is not possible to draw any definitive conclusions from such small numbers of samples, yet these results are less than encouraging.

When the HRTG data was evaluated using partial least squares regression (PLSR), rather than stepwise multiple regression, R^2 values for the validation samples were worse still with regard to their prediction accuracy (Table 7.2 in appendix).

Figure 4.3 shows characteristic NTG thermal decomposition curves for representative samples of alfalfa, bromegrass and timothy hays as well as for corresponding faecal material. It is clear from Figure 4.3 that differences were apparent between the curves of feed and faecal samples, with faecal samples tending to lose weight more slowly at low

temperatures and more rapidly and uniformly at high temperatures. Also the faecal samples demonstrated much less individual variation than was observed for the hay samples, a finding which is consistent with the chemical analysis results.

In Table 4.6 regression relationships calculated using NTG measurements conducted on faecal samples are given. Again, a strong relationship is apparent between NTG data and the measured chemical and digestibility values for the calibration samples and this time an equation with an R^2 of 0.74 was developed for DMI. Even though CP appears to be predicted relatively well using the validation samples, this and the other predictions may be meaningless, given the small numbers of samples used. The data in this table contains a number of anomalies that are probably statistical in origin, such as the high R^2 values for DEI and hemicellulose digestibility, given their low R^2 values in the calibration set and the large discrepancy between the ability to predict DMD and OMD. Also, in general SEP values were much higher with TG than with the DSC procedure used in Chapter 3.

Thermal analysis techniques necessitate the use of small sample sizes in order to prevent large temperature differences from occurring within the sample. This however, can present problems in gaining a representative sample of non-homogeneous materials such as forages. Ergudenler and Ghaly (1992) nevertheless, considered finely ground straw samples to be homogeneous, and found that sample weights similar to those used in this study were reproducible in TG studies. Most TG studies have in fact used 3-10 mg samples of lignocellulosic materials.

In conclusion, the TG analysis of 18 forages using NTG and HRTG analysis of the hay samples themselves or of faecal samples did not yield any additional information pertaining

to the lignocellulosic complex of forages and its bioavailability to cattle. Despite the reported association between the thermal degradation of lignocellulosic materials and the relationships with their quantitative and qualitative composition, no reliable evidence was found that this information is related to the animal's ability to utilise forages. Improved results may have been obtained if more samples were used, however, overall, it was considered that there was insufficient spread between individual curves for differences in the lignocellulosic components to be consistently and satisfactorily related with forage nutritive value. Differential scanning calorimetry produced curves with much greater differences and therefore appeared to be more promising. However, since DSC measurements were not useful in predicting nutritive value of forages either, further measurements with TG were not undertaken.

Table 4.1. Characteristics of alfalfa and grass hay feed samples[a]

	Calibration[c]			Prediction[d]		
Item[b]	Mean	SD[e]	Range	Mean	SD	Range
GE (MJ kg^{-1})	18.6	0.59	17.7-19.6	18.7	0.50	18.1-19.4
CP (%)	12.2	4.39	5.6-18.7	12.8	4.23	7.6-19.4
NDF (%)	63.1	6.96	51.6-76.5	60.1	15.6	41.3-76.3
ADF (%)	41.4	5.72	31.1-54.0	39.8	5.90	32.5-46.7
HEM (%)	21.7	7.36	11.0-34.8	21.0	10.1	8.4-33.0
Lignin (%)	7.3	2.49	4.3-13.1	6.6	0.85	5.2-7.7
Ash (%)	6.6	2.26	4.1-10.5	7.2	2.47	4.2-10.4
DMI (g $kg^{-0.75}$)	92.9	15.7	70-116	99.2	16.6	76-125
DEI (kJ $kg^{-0.75}$)	975	237	715-1402	1117	271	774-1582
Digestibility (%)						
DM	57.7	6.54	44.6-68.5	60.8	3.69	55.9-66.2
OM	59.2	6.36	46.7-69.8	62.4	3.89	58.1-68.5
GE	55.8	6.81	43.8-67.4	59.5	4.66	53.2-65.8
CP	59.1	8.32	47.3-75.5	61.9	14.2	42.5-78.1
NDF	54.1	7.80	41.1-65.8	56.3	10.2	40.7-68.4
ADF	48.3	5.15	40.3-57.1	50.7	6.14	43.1-58.0
HEM	64.0	12.8	36.5-79.8	64.4	19.7	32.0-85.1
DE (MJ kg^{-1})	10.4	1.34	8.5-12.9	11.1	1.05	10.0-12.6

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]Twelve samples used in calibration set.
[d]Six samples used in prediction set.
[e]Standard deviation.

Table 4.2. Characteristics of alfalfa and grass hay faecal samples[a]

	Calibration[c]			Prediction[d]		
Item[b]	Mean	SD[e]	Range	Mean	SD	Range
GE (MJ kg^{-1})	19.4	0.50	18.5-20.0	19.3	0.96	17.4-20.0
CP (%)	11.1	2.39	6.7-15.7	11.5	1.72	9.3-13.6
NDF (%)	68.1	5.19	58.3-77.0	64.8	3.63	61-70.7
ADF (%)	50.6	3.77	45.0-57.6	49.6	2.84	46.9-54.8
HEM (%)	17.5	5.38	9.7-27.0	15.2	2.97	11.1-19.1

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]Twelve samples used in calibration set .
[d]Six samples used in prediction set .
[e]Standard deviation.

Table 4.3. Simple and multiple regression relationships between feed chemical composition and nutritive value[a]

		Calibration				Prediction[e]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[f]	r^2	RMSE	R^2	SEP[g]	R^2
CP (%)	Ash, GE, lignin	3.54	0.41	2.16	0.82	1.74	0.76
NDF (%)	Ash	4.28	0.66	-	-	4.32	0.61
ADF (%)	Lignin, NDF	3.69	0.62	2.33	0.86	1.26	0.96
HEM (%)	Lignin, NDF	4.75	0.62	2.33	0.92	1.22	0.99
Lignin (%)	HEM, ADF	1.61	0.62	1.06	0.85	0.60	0.63
Ash (%)	NDF, CP	1.39	0.66	1.27	0.74	2.47	0.68
DMI (g $kg^{-0.75}$)	CP, lignin,	13.9	0.28	12.6	0.47	11.9	0.32
DEI (kJ $kg^{-0.75}$)	ADF, CP, Ash	44.7	0.43	31.3	0.78	54.7	0.55
Digestibility (%)							
DM	ADF, CP	2.61	0.85	2.35	0.89	5.67	0.02
OM	ADF, CP	2.65	0.84	2.39	0.88	5.17	0.11
GE	ADF,	2.64	0.86	-	-	6.35	0.24
CP	CP, ADF	6.07	0.51	4.94	0.71	4.64	0.82
NDF	HEM, Ash	4.49	0.70	3.78	0.81	4.20	0.82
ADF	HEM	3.74	0.52	-	-	4.34	0.42
HEM	Lignin, CP	8.19	0.56	8.26	0.66	6.63	0.07
DE (MJ kg^{-1})	ADF, GE	0.16	0.79	0.11	0.90	0.28	0.33

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to no more than three variables in equation. Missing values signify that no multiple regression equation was developed..
[e]Based on multiple regression equation developed for the calibration set.
[f]Root mean square error.
[g]Standard error of prediction.

Table 4.4. Simple and multiple regression relationships between feed normal thermogravimetric measurements (10 °C min^{-1} heating rate) including 10°C temperature changes and feed composition and nutritive value using stepwise multiple regressions.[a]

		Calibration				Prediction[e]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[f]	r^2	RMSE	R^2	SEP[g]	R^2
GE (MJ kg^{-1})	Δ240,Δ190,Δ450	0.35	0.41	0.06	0.85	0.19	0.02
CP (%)	Δ390,Δ200,Δ350	2.86	0.61	1.94	0.86	4.39	0.18
NDF (%)	Δ410	5.03	0.52	-	-	5.55	0.32
ADF (%)	Δ350,Δ290,Δ330	4.04	0.55	1.84	0.92	4.40	0.33
HEM (%)	320,Δ350,Δ410	6.36	0.32	4.65	0.71	8.95	0.20
Lignin (%)	Δ350,430,Δ330	1.44	0.70	1.17	0.84	1.59	0.13
Ash (%)	Δ390,Δ400,Δ490	1.23	0.73	0.73	0.92	1.46	0.40
DMI (g $kg^{-0.75}$)	None	-	-	-	-	-	-
DEI (kJ $kg^{-0.75}$)	Δ250	46.2	0.40	-	-	88.2	0.00
Digestibility (%)							
DM	Δ250,460,Δ420	4.39	0.59	2.29	0.91	9.62	0.02
OM	450,Δ360,Δ250	4.26	0.59	2.24	0.91	10.9	0.00
GE	270,Δ240,Δ340	4.42	0.62	2.62	0.89	8.70	0.03
CP	Δ390,380	6.86	0.38	5.26	0.67	10.6	0.36
NDF	Δ340,160,Δ430	6.45	0.38	3.89	0.82	7.95	0.22
ADF	310,220,Δ440	3.99	0.45	3.14	0.73	11.1	0.00
HEM	Δ340,160,Δ280	9.00	0.55	3.61	0.94	4.96	0.75
DE (MJ kg^{-1})	270,Δ240,Δ340	0.22	0.57	0.01	0.93	0.29	0.12

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]Components comprise actual temperatures or the 10°C change in temperature recorded at this temperature. First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to no more than three variables in equation. Missing values signify that no multiple regression equation was developed.
[e]Based on multiple regression equation developed for the calibration set.
[f]Root mean square error.
[g]Standard error of prediction.

Table 4.5. Simple and multiple regression relationships between feed high resolution thermogravimetric measurements (50 °C min^{-1} heating rate) including 10 °C temperature changes and feed composition and nutritive value using stepwise multiple regressions.[a]

		Calibration				Prediction[e]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[f]	r^2	RMSE	R^2	SEP[g]	R^2
GE (MJ kg^{-1})	Δ410,Δ170,Δ570	0.12	0.32	0.07	0.82	0.32	0.14
CP (%)	330	2.08	0.80	-	-	2.5	0.72
NDF (%)	Δ250,Δ480,Δ540	4.22	0.66	2.17	0.93	9.77	0.32
ADF (%)	Δ560,Δ370,Δ600	4.42	0.46	3.05	0.79	7.64	0.44
HEM (%)	Δ480,Δ410,Δ600	5.37	0.52	3.12	0.87	11.0	0.11
Lignin (%)	Δ530,Δ170,Δ240	1.81	0.52	1.30	0.80	3.27	0.24
Ash (%)	420,Δ170,Δ250	1.03	0.81	0.65	0.94	2.39	0.32
DMI (g $kg^{-0.75}$)	Δ590,Δ430,Δ500	10.9	0.56	4.98	0.93	18.1	0.00
DEI (kJ $kg^{-0.75}$)	Δ590,Δ540	47.4	0.37	34.7	0.69	61.9	0.08
Digestibility (%)							
OM	Δ530,Δ280,Δ440	4.15	0.63	2.49	0.89	11.6	0.01
DM	Δ530,Δ280,Δ440	4.06	0.63	2.46	0.89	11.7	0.01
GE	Δ530,Δ370,Δ590	4.60	0.59	2.72	0.88	9.71	0.08
CP	Δ500,Δ250,Δ570	4.48	0.74	1.56	0.97	9.43	0.54
NDF	Δ530,250,Δ220	6.21	0.42	4.21	0.79	9.88	0.08
ADF	Δ600,Δ580,Δ530	3.50	0.58	2.11	0.88	4.24	0.08
HEM	Δ530,190,Δ410	11.4	0.28	6.31	0.82	9.55	0.02
DE (MJ kg^{-1})	Δ540,Δ370,Δ530	0.21	0.63	0.14	0.86	0.37	0.02

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]Components comprise actual temperatures or the 10°C change in temperature recorded at this temperature. First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to no more than three variables in equation. Missing valuessignify that no multiple regression equation was developed.
[e]Based on multiple regression equation developed for the calibration set.
[f]Root mean error square.
[g]Standard error of prediction.

Table 4.6. Simple and multiple regression relationships between faecal normal thermogravimetric measurements (10 °C min^{-1} heating rate) including 10 °C temperature changes and feed composition and nutritive value using stepwise multiple regression.[a]

		Calibration				Prediction[e]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[f]	r^2	RMSE	R^2	SEP[g]	R^2
GE (MJ kg^{-1})	290	0.11	0.25	-	-	0.02	0.00
CP (%)	440,200,520	1.62	0.58	0.98	0.88	0.54	0.75
NDF (%)	Δ480,Δ200,Δ350	3.66	0.55	1.81	0.91	2.57	0.13
ADF (%)	Δ250,Δ300	2.98	0.43	2.59	0.61	3.58	0.00
HEM (%)	Δ430,Δ440,Δ450	3.12	0.69	1.67	0.93	4.00	0.39
DMI (g $kg^{-0.75}$)	530,540,Δ290	14.1	0.27	9.33	0.74	17.7	0.24
DEI (kJ $kg^{-0.75}$)	Δ300	52.2	0.23	-	-	4.78	0.87
Digestibility (%)							
DM	Δ410,Δ240,Δ460	4.39	0.59	2.47	0.90	7.47	0.13
OM	Δ410,Δ240,Δ460	4.24	0.60	2.44	0.89	5.52	0.47
GE	Δ410,440,Δ210	4.69	0.57	3.10	0.85	2.10	0.36
CP	530,Δ180,Δ450	6.61	0.42	4.44	0.79	10.5	0.69
NDF	250,Δ280,Δ300	6.05	0.45	3.92	0.82	5.71	0.06
ADF	260	3.41	0.60	-	-	4.34	0.00
HEM	Δ450	9.55	0.49	-	-	1.68	0.73
DE (MJ kg^{-1})	Δ410,440,470	0.25	0.47	0.19	0.74	0.20	0.29

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]Components comprise actual temperatures or the 10°C change in temperature recorded at this temperature. First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to no more than three variables in equation. Missing values signify that no multiple regression equation was developed.
[e]Based on multiple regression equation developed for the calibration set.
[f]Root mean error square.
[g]Standard error of prediction.

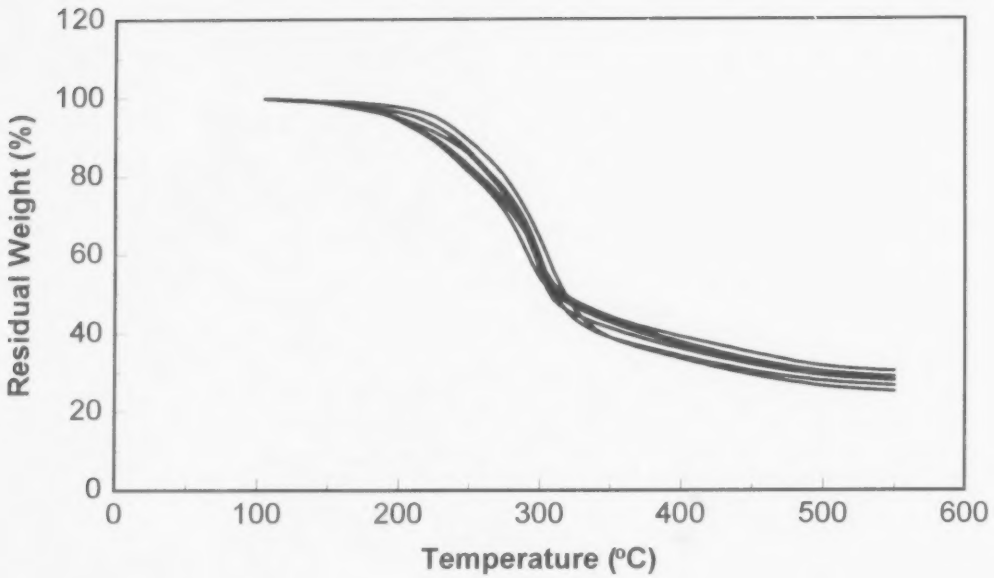

Figure 4.1. High resolution TG curves of seven alfalfa samples.

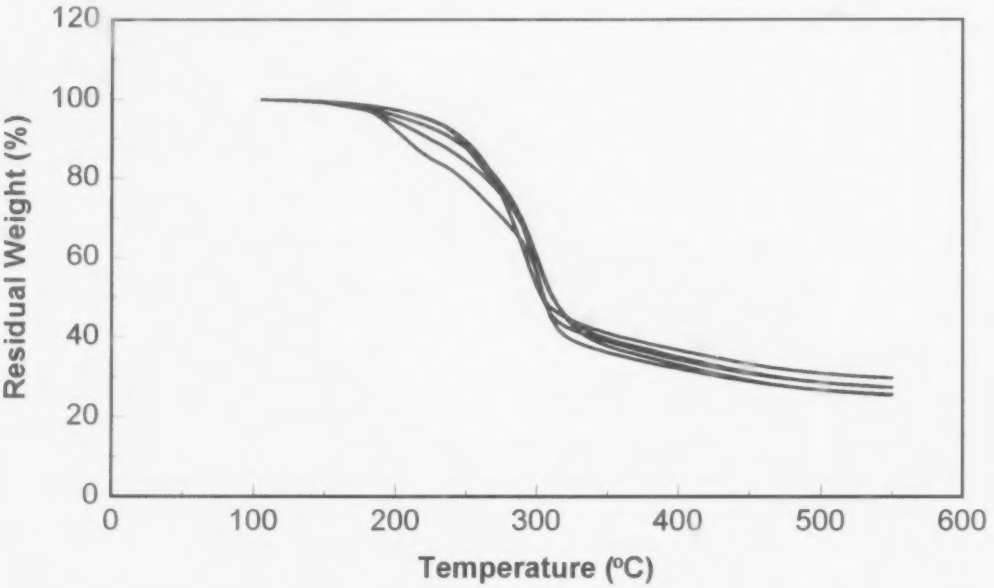

Figure 4.2. High resolution TG curves of five bromegrass samples.

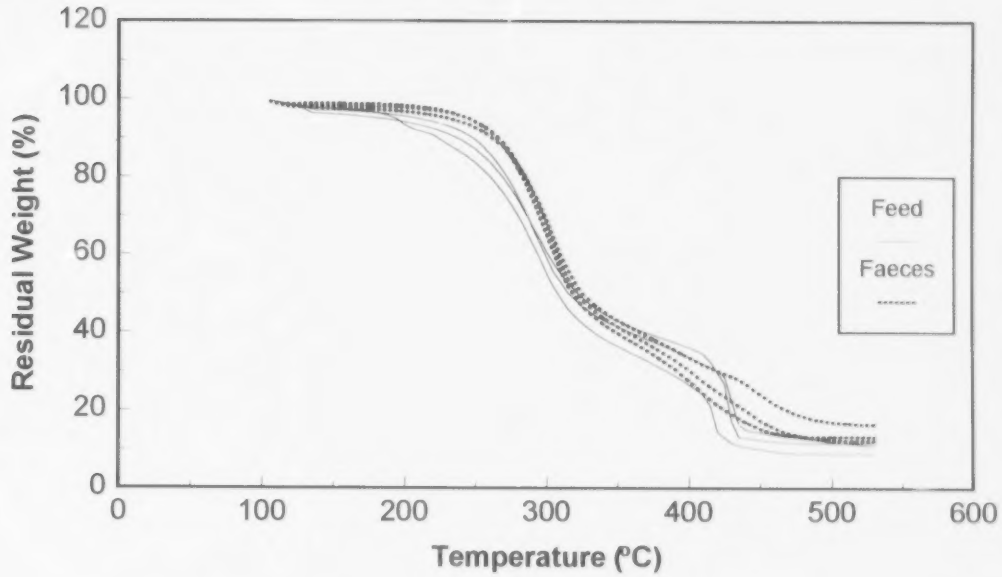

Figure 4.3. Normal TG curves of one sample each of alfalfa, bromegrass and timothy hay and faeces.

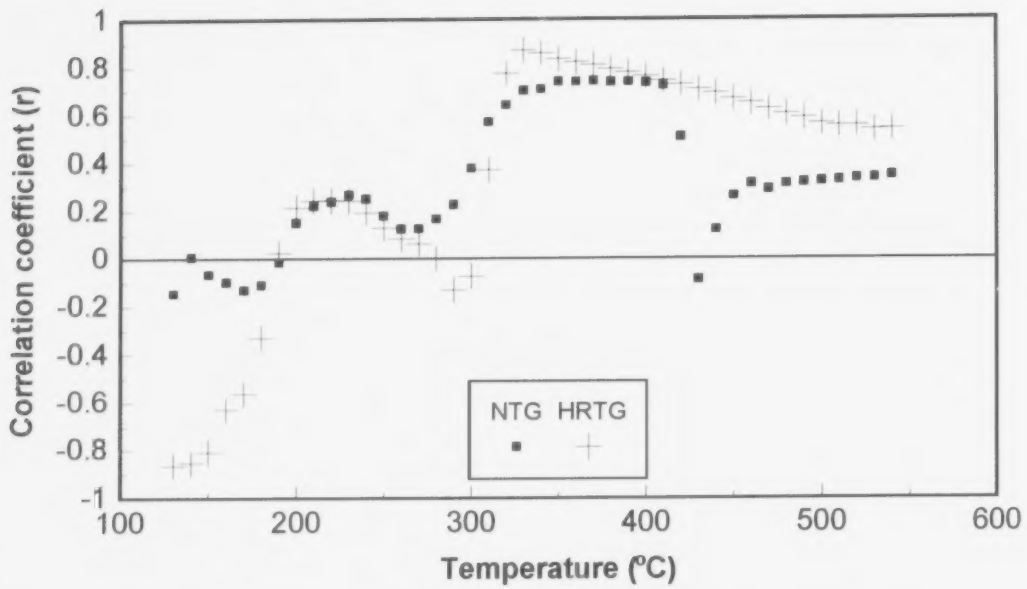

Figure 4.4. Correlation between crude protein and normal and high resolution TG residual weights at each temperature for all 18 hay samples.

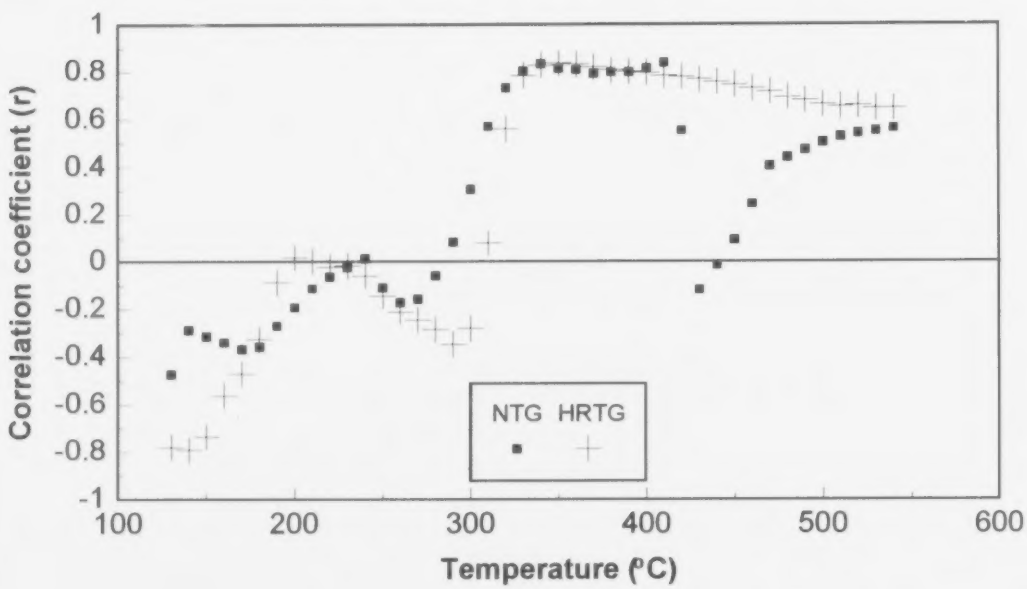

Figure 4.5. Correlation between ash and normal and high resolution TG residual weights at each temperature for all 18 hay samples.

5.0 GENERAL DISCUSSION AND CONCLUSIONS

Given the impracticality and expense of conducting in-vivo feeding trials to evaluate large numbers of samples, reliable laboratory procedures are required that can adequately predict animal response. Unfortunately, due to the complexity of the problem and our incomplete understanding of forage biodegradability in the rumen, laboratory procedures that will accurately predict the nutritive value of forages still elude ruminant nutritionists. The purpose of this study was therefore to determine whether the thermal analysis techniques of differential scanning calorimetry (DSC) and thermogravimetry (TG) can be used to estimate the chemical composition and nutritive value of grass and legume hays. Norris et al. (1976) were the first authors to apply a similar multivariate analysis procedure (NIRS) to the prediction of forage quality. Some thermal analysis techniques can also record a whole spectrum of chemical information directly from each sample and since the curves generated have some similarities to NIRS curves, we had believed that the thermal techniques might also have potential for forage analysis. In addition, techniques such as thermal analysis eliminate the risk of introducing spurious artefacts into traditional analysis by way of the chemical reagents used during extraction procedures, a limitation of chemical procedures as discussed by Reh et al. (1986). To our knowledge, no one has tried using thermal analysis procedures for estimating forage quality previously.

Chapter 3 reports the results of the main study whereby 93 assorted hay samples, for which chemical composition and digestibility data were available, were analysed using DSC. The results of a very preliminary study of a subsample of 18 of these hays by NTG and

HRTG are outlined in Chapter 4. The thermal curves were then used to predict the chemical composition and nutritive value of the forages concerned. The methodology employed in this study to handle the data is analogous to that reported for evaluating NIRS spectral data by other workers (Norris et al.,1976; Redshaw et al.,1986).

In the DSC study, percentage CP and NDF were the parameters most accurately estimated from the validation samples, having R^2 values of 0.61 and 0.72 respectively, but these values are inferior to those achieved using NIRS by other workers (Norris et al.,1976; Redshaw et al.,1986; Martin and Linn,1989; Snyman and Joubert,1993). Relationships between other chemical fractions of interest and DSC values were either moderate or poor (R^2=0.34, 0.01 0.46 for ADF, lignin and ash respectively). Similarly, relationships between cattle intake and digestibility data were not good (R^2 =0.13 for both DMI and DMD). Analysis of the same data using partial least squares regression (PLSR), as opposed to multiple stepwise regression produced very similar results, although neither yielded good relationships.

When different forage samples were analysed using NTG and HRTG techniques, predictions of chemical composition and nutritive value were either poor or inconsistent. Similarly, analysis of faecal samples using NTG was not useful. It was deemed that differences in the lignocellulosic components of these samples would not be distinguished adequately, since there was insufficient spread between the individual curves. Therefore, since DSC measurements were not useful in predicting forage nutritive value, and thermal curves obtained from DSC demonstrated a much greater spread than those obtained from TG, further TG measurements were not undertaken. In the TG study only a very small number

of samples were assessed. The study was however, purely exploratory, to determine if differences between forage samples were detectable and provide some indication of whether it had any potential. For this technique, the 18 prepared samples were analysed elsewhere since no TG equipment was available locally.

A number of inherent limitations are associated with the analysis of forages using thermal analysis equipment. The most fundamental of these is the necessity for small samples sizes, in the order of a few mg, which presents problems in gaining representative samples from heterogeneous materials. In spite of this limitation, thermal curves of feeds, according to our data, can be reproduced with acceptable accuracy over the majority of the curve although the accuracy of DSC duplicate runs during the final peak of thermal degradation may also be a concern. Also, thermal analysis techniques do not have the same potential as NIRS for routine forage evaluation programmes, due to the time requirement for analysis (around 65 min for DSC with a heating rate of $5^{o}C\ min^{-1}$).

There were potential problems associated with our DSC and TG studies which may have reduced the predictive ability of the procedure. The database of samples we used were six to twelve years old and although these samples were dry and stored in sealed containers at low temperatures, it is not known if they had deteriorated markedly over time. Workers assessing the NIRS technique (Norris et al.,1976; Redshaw et al.,1986) have, however used forage samples stored for a comparable time period.

Even if the thermal degradation of the lignocellulose components of forages, as determined either by DSC or TG had been closely related in an absolute manner to chemical analysis or animal response data, this system is subject to the same inherent problems

associated with any predictive regression technique. An important limitation with the technique is that it was empirical rather than analytical, thus absolute accuracy is unattainable. In addition, the empirical approach to forage evaluation requires the compilation of large reference data sets and these are currently being established worldwide for the NIRS procedure. Redshaw et al. (1986) pointed out that the multivariate approach incorporates not only variation arising from the independent (NIRS) measurements themselves, but also variations in the calibration data. When this data includes animal related measurements, between animal variability is included in the resultant predictions. These authors estimated that such variability in the calibration data accounted for 25% and 50% respectively, of the variability in NIRS prediction of DMD and voluntary DMI in cattle. Standard errors associated with cattle data, reported by Redshaw et al. (1986), were 3.6 g $kg^{-0.75}$ for DMI, 0.59% for DDM, 0.11 MJ kg^{-1} for DE and 38 kJ $kg^{-0.75}$ for DEI. Similar levels of animal variation can be assumed for the cattle data in our experiment.

Mean differences between duplicate samples using DSC were approximately 1.3 mW, some of which may be attributable to sampling errors. Although mean differences between curves were in the 2 to 5% range, relatively large differences (4 to 12%) between duplicates were obtained in the 430 to 470°C temperature range. This region of the curve was used in the prediction of ADF, DMD, OMD, GE digestibility, and DE. A major limitation of this technique, then, is the lack of precision of measurements in this temperature region.

Many other workers have used DSC to study thermal transitions occurring under high moisture/low temperature conditions such as starch gelatinisation and protein denaturation. It may be that addition of moisture prior to the analysis of forage material using DSC would

have yielded additional information pertaining to molecular changes occurring in the cell wall components in this environment as a consequence of heating. Only one study using this type of approach was found in the literature and this used DSC to examine pectin/calcium interactions in cell wall preparations isolated from elongating and mature regions of soybean hypocotyls (Lin et al.,1991). Additions of calcium significantly altered the thermal transitions of the young, but not the mature cell wall preparations and it was considered that calcium binding to pectin and strengthening the cell wall may account for this observed change. This approach is interesting and warrants further examination.

In conclusion, thermal analysis does not appear to have potential for estimating the nutritive value of forages since it is a time consuming procedure, can only accommodate very small sample sizes and and our results demonstrate that it could not adequately estimate a range of forage chemical composition and cattle intake and digestibility parameters. The NIRS technique has previously been shown to achieve much better results than we were able to obtain with thermal analysis techniques in this experiment. However we do believe that multivariate approaches to the prediction of nutritive value are required. The search is still on to develop better forage quality evaluation procedures that have a sound molecular basis and can be directly related to chemical or physical properties of the actual forage plant.

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7.0 APPENDIX

Table 7.1. Partial least squares regression analysis of feed differential scanning calorometry measurements (5°C min^{-1} heating rate) and feed chemical analysis data using NIRS[g] software[a]

	Calibration			Prediction[e]		
Item[b]	n[c]	SEC[d]	R^2	n	SEP[f]	R^2
GE (MJ kg^{-1})	55	0.09	0.17	37	0.11	0.02
CP (%)	54	2.14	0.70	37	2.35	0.50
NDF (%)	53	3.21	0.92	37	5.05	0.73
ADF (%)	52	2.60	0.69	37	6.45	0.09
HEM (%)	53	3.44	0.81	37	6.53	0.27
Lignin (%)	49	1.32	0.39	37	2.50	0.01
Ash (%)	53	0.82	0.82	37	1.52	0.49
ADIN (%)	52	0.05	0.53	37	0.09	0.20
DMI (g $kg^{-0.75}$)	54	11.5	0.58	37	14.2	0.22
DEI (kJ $kg^{-0.75}$)	56	43.6	0.41	37	44.0	0.28
Digestibility (%)						
DM	53	2.73	0.26	37	4.55	0.16
OM	51	3.19	0.14	37	4.64	0.18
GE	53	2.93	0.35	37	4.79	0.18
CP	54	5.79	0.67	37	7.97	0.45
NDF	55	6.76	0.42	37	6.24	0.27
ADF	54	5.48	0.16	37	4.30	0.31
DE (MJ kg^{-1})	55	0.18	0.28	37	0.21	0.15

[a]In this experiment 93 hays were examined.
[b]Abbreviations used:ADF=acid detergent fibre, ADIN=acid detergent insoluble nitrogen, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]Number of samples used to develop the calibration equation for each variable.
[d]Standard error of calibration.
[e]Independent sample set..
[f]Standard error of prediction.
[g]Near infrared reflectance spectroscopy.

Table 7.2. Partial least squares regression analysis of feed high resolution thermogravimetric measurements (50 °C min^{-1} heating rate) and feed chemical analysis data using NIRS[g] software.[a]

	Calibration			Prediction[e]		
Item[b]	n[c]	SEC[d]	R^2	n	SEP[f]	R^2
GE (MJ kg^{-1})	12	0.02	0.97	6	0.18	0.02
CP (%)	12	1.60	0.87	6	4.47	0.01
NDF (%)	12	2.54	0.87	6	14.1	0.10
ADF (%)	12	3.10	0.71	6	7.11	0.38
HEM (%)	12	4.02	0.7	6	8.6	0.14
Lignin (%)	11	0.66	0.86	6	1.4	0.07
Ash (%)	12	0.71	0.9	6	2.66	0.06
DMI (g $kg^{-0.75}$)	12	8.62	0.7	6	17.6	0.24
DEI (kJ $kg^{-0.75}$)	12	28.7	0.74	6	88.4	0.16
Digestibility (%)						
DM	12	3.77	0.67	6	7.65	0.00
OM	12	3.58	0.68	6	7.89	0.02
GE	12	3.70	0.71	6	8.68	0.04
CP	12	0.90	0.99	6	14.2	0.03
NDF	12	4.94	0.6	6	8.7	0.27
ADF	12	2.84	0.7	6	6.68	0.24
HEM	12	8.45	0.56	6	17	0.15
DE (MJ kg^{-1})	12	0.16	0.75	6	0.44	0.01

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used:ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible energy intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre, and OM=organic matter.
[c]Number of samples used to develop the calibration equation for each variable.
[d]Standard error of calibration.
[e]Independent sample set.
[f]Standard error of prediction.
[g]Near infrared reflectance spectroscopy.

Table 7.3. Simple and multiple regression relationships between feed normal thermogravimetric measurements (10 °C min^{-1} heating rate) and feed composition and nutritive value using stepwise multiple regressions[a]

		Calibration				Prediction[e]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[f]	r^2	RMSE	R^2	SEP[g]	R^2
CP (%)	390	3.22	0.51	-	-	2.24	0.65
NDF (%)	180, 290, 460	5.38	0.45	-	0.74	13.2	0.02
ADF (%)	450	4.09	0.53		-	5.42	0.20
HEM (%)	320	6.36	0.32	-	-	4.74	0.29
Lignin (%)	450 ,180, 310	1.89	0.48	1.26	0.81	5.86	0.14
Ash (%)	410, 370, 420	1.26	0.72	0.75	0.91	1.94	0.54
DMI (g $kg^{-0.75}$	None	-	-	-	-	-	-
DEI (kJ $kg^{-0.75}$	None	-	-	-	-	-	-
Digestibility (%)							
DM	450, 160	4.39	0.59	3.93	0.70	9.9	0.00
OM	450, 160	4.26	0.59	3.79	0.70	9.49	0.01
GE	270, 230, 450	4.42	0.62	2.98	0.86	9.11	0.01
CP	170	7.32	0.29	-	-	3.92	0.05
NDF	320, 340, 430	6.76	0.32	5.00	0.70	15.6	0.01
ADF	310	4.02	0.45	-	-	6.16	0.01
HEM	None	-	-	-	-	-	-
DE (MJ kg^{-1})	270, 230, 450	0.22	0.57	0.16	0.81	0.42	0.04

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible enery intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre and OM=organic matter.
[c]Components comprise actual temperatures. First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to no more than three variables in equation and missing values signify that no regression equation was developed.
[e]Based on multiple regression equation developed for the calibration set.
[f]Root mean error square.
[g]Standard error of prediction.

Table 7.4. Simple and multiple regression relationships between faecal normal thermogravimetric measurements (10 °C min^{-1} heating rate) and feed composition and nutritive value using stepwise multiple regressions.[a]

		Calibration				Prediction[e]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[f]	r^2	RMSE	R^2	SEP[g]	R^2
CP (%)	440, 430, 510	1.62	0.58	1.04	0.86	0.57	0.73
NDF (%)	460, 330	3.69	0.54	3.14	0.70	3.66	0.55
ADF (%)	250	2.98	0.43	-	-	2.88	0.01
HEM (%)	440, 410, 450	3.84	0.54	2.06	0.89	4.43	0.19
Lignin (%)	None	-	-	-	-	-	-
Ash (%)	None	-	-	-	-	-	-
DMI (g $kg^{-0.75}$	540	14.1	0.26	-	-	13.8	0.12
DEI (kJ $kg^{-0.75}$	None	-	-	-	-	-	-
Digestibility (%)							
DM	270	4.92	0.49	-	-	1.54	0.80
OM	270	4.78	0.49	-	-	1.54	0.79
GE	270	4.81	0.55	-	-	2.15	0.68
CP	530	6.61	0.42	-	-	4.37	0.80
NDF	250	6.07	0.45	-	-	6.06	0.02
ADF	250	3.46	0.59	-	-	4.61	0.00
HEM	320, 240, 280	11.6	0.25	9.45	0.60	8.05	0.20
DE (MJ kg^{-1})	270	0.25	0.46	-	-	0.08	0.73

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible enery intake, DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre and OM=organic matter.
[c]Components comprise actual temperatures. First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to no more than three variables in equation and missing values signify that no regression equation was developed.
[e]Based on multiple regression equation developed for the calibration set.
[f]Root mean error square.
[g]Standard error of prediction.

Table 7.5. Simple and multiple regression relationships between feed high resolution thermogravimetric measurements (50 °C min^{-1} heating rate) and feed composition and nutritive value using stepwise multiple regressions.[a]

		Calibration				Prediction[e]	
		Simple		Multiple[d]			
Item[b]	Components[c]	RMSE[f]	r^2	RMSE	R^2	SEP[g]	R^2
CP (%)	330	2.08	0.80	-	-	2.50	0.72
NDF (%)	450, 210, 250	5.19	0.49	3.13	0.85	4.35	0.76
ADF (%)	250	5.02	0.30	-	-	1.56	0.81.
HEM (%)	440	6.12	0.37	-	-	5.57	0.29
Lignin (%)	None	-	-	-	-	-	-
Ash (%)	420	1.03	0.81	-	-	2.30	0.36
DMI (g $kg^{-0.75}$	320	13.5	0.32	-	-	8.26	0.31
DEI (kJ $kg^{-0.75}$	160	50.9	0.27	-	-	42.1	0.36
Digestibility (%)							
DM	240	5.53	035	-	-	4.34	0.00
OM	240	5.39	0.35	-	-	4.09	0.05
GE	240	5.40	0.43	-	-	4.90	0.04
CP	330, 180	6.29	0.48	4.23	0.81	13.2	0.48
NDF	None	-	-	-	-	-	-
ADF	None	-	-	-	-	-	-
HEM	None	-	-	-	-	-	-
DE (MJ kg^{-1})	240, 320	0.27	0.39	0.23	0.59	0.52	0.09

[a]Seven alfalfa hays and 11 grass hays were examined in the experiment.
[b]Abbreviations used: ADF=acid detergent fibre, CP=crude protein, DE=digestible energy, DEI=digestible enery intake,DM=dry matter, DMI=dry matter intake, GE=gross energy, HEM=hemicellulose, NDF=neutral detergent fibre and OM=organic matter.
[c]Components comprise actual temperatures. First component listed is the one which was used in the simple regression.
[d]Multiple regression limited to no more than three variables in equation and missing values signify that no regression equation was developed.
[e]Based on multiple regression equation developed for the calibration set.
[f]Root mean error square.
[g]Standard error of prediction.

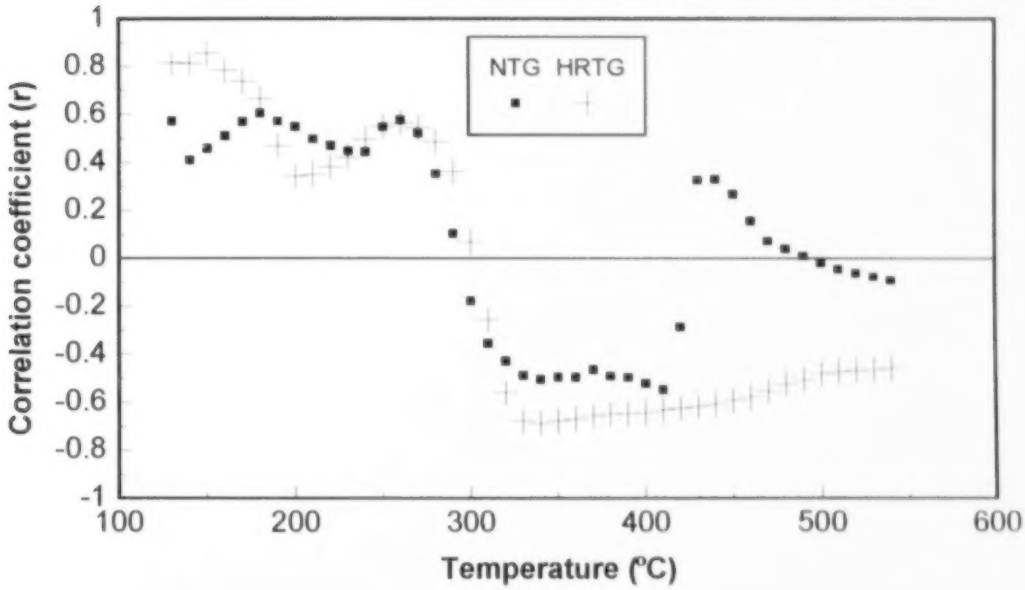

Figure 7.1. Correlation between neutral detergent fibre and normal and high resolution TG residual weights at each temperature for all 18 hay samples.

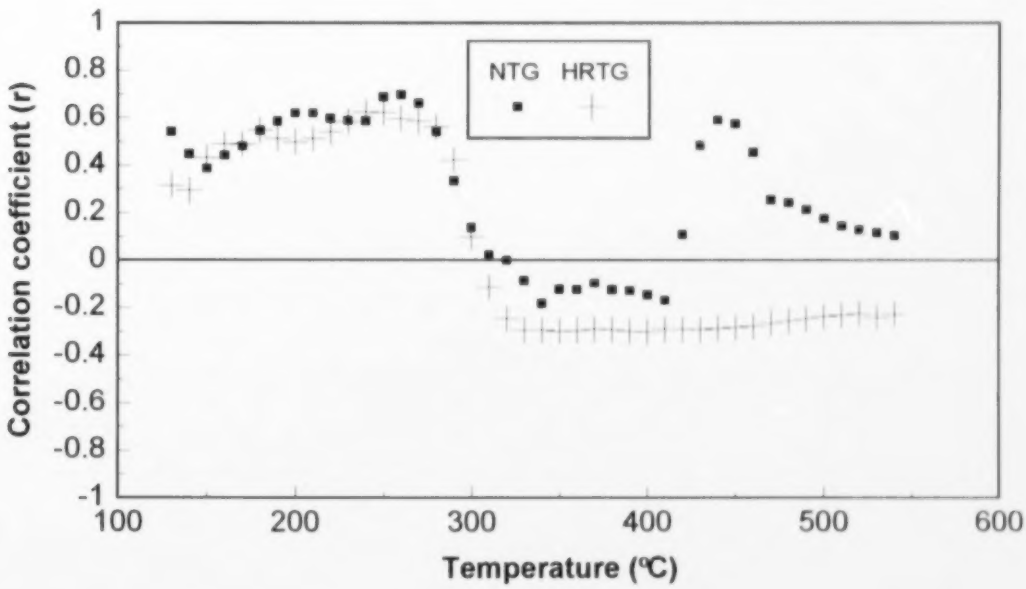

Figure 7.2. Correlation between acid detergent fibre and normal and high resolution TG residual weights at each temperature for all 18 hay samples.

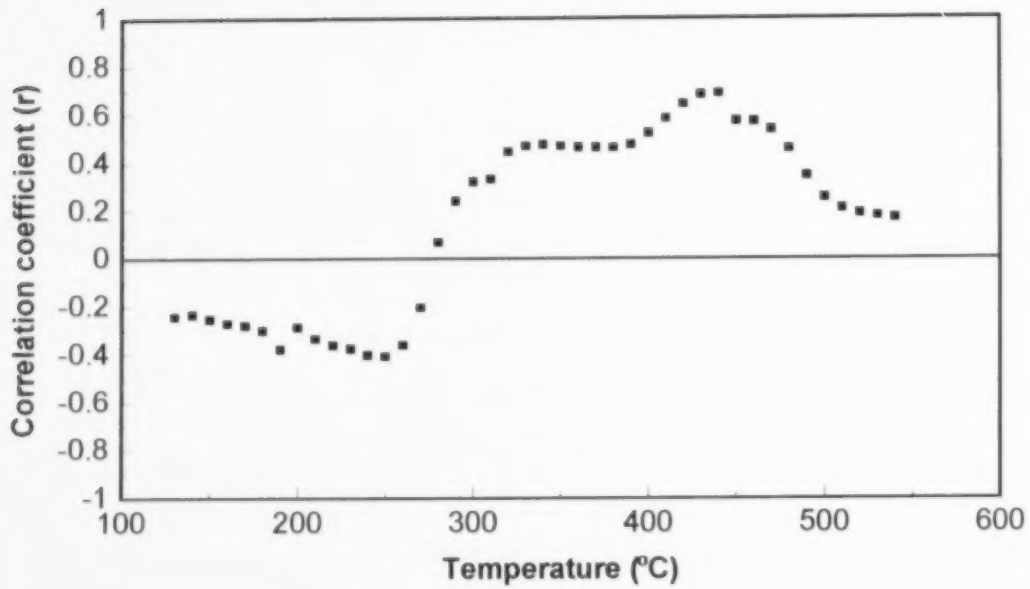

Figure 7.3. Correlation between crude protein and normal TG residual weights at each temperature for all 18 faecal samples.

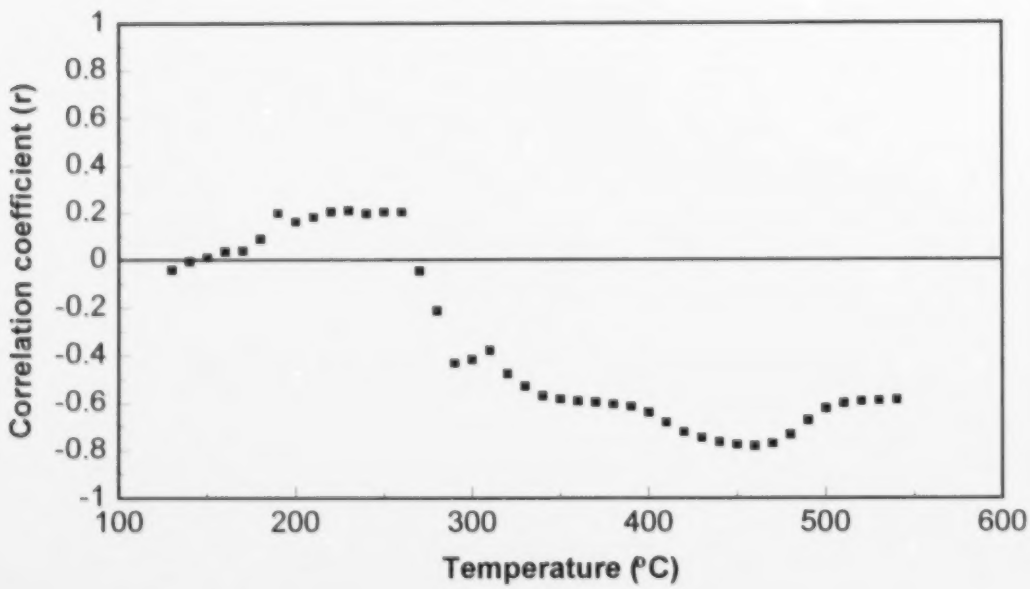

Figure 7.4. Correlation between neutral detergent fibre and normal TG residual weights at each temperature for all 18 faecal samples.

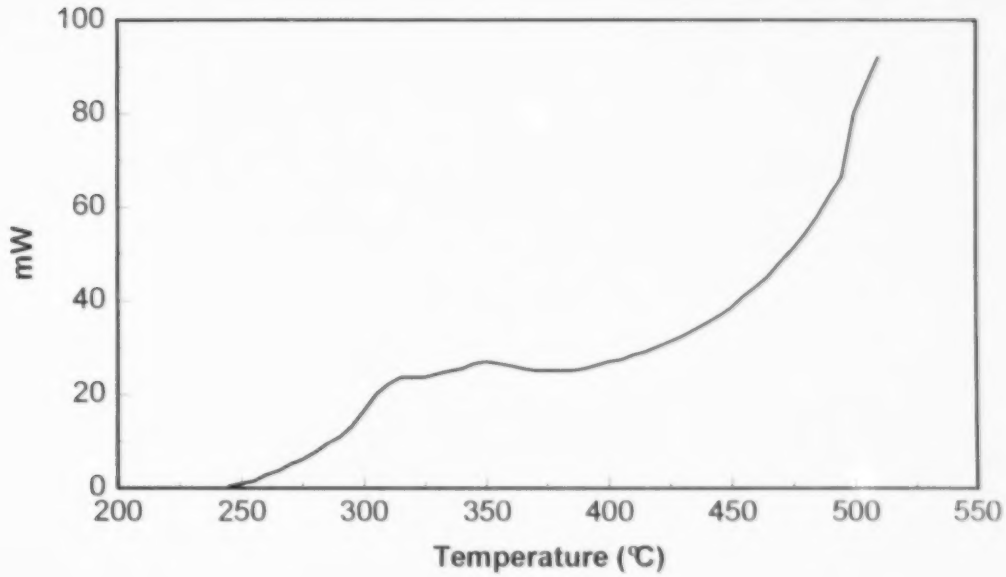

Figure 7.5. Differential scanning calorimetry curve of purified bovine casein.